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variation
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TEXTBOOK OF PRACTICAL BOTANY

By the same authors

TEXTBOOK OF THEORETICAL BOTANY

TEXTBOOK OF PRACTICAL BOTANY

BY

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PREFACE

In every science it is true to say that the quality of the laboratory work is at least as important as the quality of the theoretical teaching, though a high standard in the former is much more difficult to reach than in the latter. Among the factors which militate against success, so far as Botany is concerned, is the lack of guides to the nature and scope of laboratory work at different grades. There exist theoretical textbooks and monographs of high quality, but there is a scarcity of books on practical studies of a sufficiently comprehensive kind to match the needs of modern teachers.

This book gives in detail the work suitable for a general University course. The descriptions of all the material examined are original, being taken from actual selected specimens, so as to express just what may be seen. In certain particulars, here and there, they differ from the traditional accounts, where observation has shown this to be necessary. The authors hope that their accuracy will be proven by the experience of others.

The anatomical and morphological descriptions given in the text are, with few exceptions, limited to mature structures, which are readily available to students. For the same reason the gametophyte generation of several of the Pteridophyte types have been omitted, as they are only very rarely accessible. The number of types described, especially of the Thallophyta, has been strictly limited to those which were regarded as essential.

The illustrations are almost entirely photographic, and they have been limited to objects which are difficult, either to obtain, or to prepare for examination. This may help some people over hurdles, and at the same time show the more fortunate student what to expect, without giving him pictures which he can idly copy.

Quantitative measurements have been introduced into the experimental sections, wherever they seemed useful. Experience shows that it is very necessary to impress the student of Botany with the need for accurate measurements, as there is a decided tendency in student quarters to regard Botany as simply descriptive and qualitative, in contrast to the " exact " sciences. This is a serious error and should be strenuously combated.

The book also goes outside purely morphological and physiological limits and deals with the type of ecological work which can be best done by a class in the laboratory. Field work in Ecology, indispensable though it is, we have only touched upon in outline, as a full treatment of the subject has been given in "Practical Field Ecology," by the present authors.

A section on Variation and Heredity is included as an introduction to this very important branch of study. It may be criticised as too brief, but we believe that even the simplest introduction to practical work gives reality to an otherwise highly theoretical subject.

The arrangement of the text is fully set out in the Table of Contents, and for this reason the Subject Index is only of limited extent and is intended to supplement the Table of Contents. An Index to the names of plants mentioned in the text is included.

The nomenclature of British Plants used in the text is that of the "Check List of British Plants," published in the *Journal of Ecology*, 1946.

The present work is intended to be used with the authors' "Textbook of Theoretical Botany," which will be completed in four volumes. Although it will be obvious that the two works could not be co-extensive, the student who has carried out the practical work here described should have a sufficient training to enable him to cope with the more comprehensive laboratory studies required by a full University course.

R. C. McLEAN.
W. R. IVIMEY-COOK.

UNIVERSITY OF WALES,
UNIVERSITY COLLEGE, CARDIFF.
March 1950.

POSTSCRIPT

WHEN the book was in page proof early this year, my colleague Dr Ivimey-Cook died. At this time I was in the Far East and my best thanks are due to Dr B. Barnes for his kindness in reading the page proofs. Dr Barnes, however, must not be held responsible for any material errors that may occur.

R. C. McLEAN.

UNIVERSITY COLLEGE,
CARDIFF.
May 1952.

TABLE OF CONTENTS

PAGE
LIST OF ILLUSTRATIONS xix

OUTLINE CLASSIFICATION OF PLANTS xxii

PART I.—METHODS

SECTION 1. OBJECTS AND METHODS OF LABORATORY STUDY

A. History 1
B. Scope of Laboratory Studies 2
C. Principal Methods 3
D. Necessary Materials 3

SECTION 2. THE MICROSCOPE

A. Purchasing a Microscope 4
B. Essentials of the Microscope 5
C. Accessories 6
D. Using the Microscope 6
E. Measurement 7
F. Drawing 8

SECTION 3. METHODS AND REAGENTS

A. Preparation of Material 9
  1. Fixing 10
  2. Hardening 11
  3. Preserving 11
  4. Maceration 11
  5. Mounts of Entire Objects 12
B. Section Cutting 12
C. Staining Methods 15
  1. Histological Stains 17
  2. Cytological Stains 20
  3. Stains for Thallophytes 23
  4. Stains for Special Purposes 24

D. Mounting Preparations

  1. Mounting Media 25
  2. Ringing Slides 26
  3. Labelling 26

E. Embedding and Microtome Technique

  1. Embedding 27
  2. Microtomy 28

DIAGRAMMATIC SCHEME OF THE STAINING PROCESSES FOR SECTIONS . . 30

PART II.—CYTOLOGY

SECTION 1. ELEMENTS OF CELL STRUCTURE PAGE
A. The Mature Cell
1. Cell Wall 31
2. Living Cells 32
B. Protoplasm and the Meristematic Cell
1. Cyclosis 33
2. Protoplasmic Connections 34
3. Plasmolysis 35
4. Meristems 35
C. Nuclear Division 36

SECTION 2. CELL INCLUSIONS AND RESERVES
A. Chloroplasts 39
B. Chromoplasts 40
C. Anthocyanins 40
D. Leucoplasts 41
E. Starch Grains 41
F. Inulin 42
G. Cell Crystals 43
H. Aleurone Grains 45

SECTION 3. MICROCHEMICAL REACTIONS 45
A. Constructional Materials 46
B. Cell Contents 48

SECTION 4. TYPES OF MATURE CELLS AND ELEMENTARY TISSUES
A. Parenchyma 50
B. Collenchyma 51
C. Sclereids 51
D. Sclerenchyma 52
E. Stelar Elements 52

PART III.—EVOLUTIONARY MORPHOLOGY

SECTION 1. ALGAE
A. Chlorophyceae
1. Chlamydomonas 54
2. Haematococcus (Sphaerella) 55
3. Pandorina 56
4. Eudorina 56
5. Volvox 58
6. Ulothrix 59
7. Pleurococcus 59
8. Spirogyra 60
9. Vaucheria 61
10. Oedogonium 63
B. Cyanophyceae
1. Nostoc 65

PAGE
C. Rhodophyceae
1. Batrachospermum 65
2. Chondrus 66
3. Polysiphonia 67
4. Corallina 69
D. Phaeophyceae
1. Ectocarpus 71
2. Cutleria 72
3. Dictyota 74
4. Laminaria 75
5. Fucus 78

SECTION 2. FUNGI

NOTES ON THE PREPARATION OF AGAR 80
A. Archimycetes
1. Plasmodiophora 81
2. Fuligo 82
3. Arcyria 83
4. Reticularia 83
B. Phycomycetes
1. Mucor 84
2. Saprolegnia 86
3. Monoblepharis 87
4. Pythium 89
5. Cystopus 90
6. Peronospora 91
C. Ascomycetes
1. Aspergillus 92
2. Erysiphe 93
3. Peziza 94
4. Rhytisma 95
5. Saccharomyces 96
6. Claviceps 97
7. Hypoxylon 98
D. Basidiomycetes
1. Puccinia 99
2. Polyporus 102
3. Psalliota 102
4. Cyathus 104
5. Phallus 105

SECTION 3. BACTERIA
1. Bacillus 106
2. Staphylococcus 107

SECTION 4. CHARALES
1. Chara 108

SECTION 5. HEPATICAE
1. Pellia 109
2. Marchantia 112
3. Anthoceros 115

PAGE

SECTION 6. MUSCI

1. Mnium 117
2. Funaria 122
3. Polytrichum 124
4. Sphagnum 129

SECTION 7. FILICALES

1. Dryopteris 131
2. Pteridium 139
3. Hymenophyllum 142
4. Osmunda 143
5. Botrychium 144
6. Azolla 145

SECTION 8. EQUISETALES

1. Equisetum 149

SECTION 9. LYCOPODIALES

1. Lycopodium 154
2. Selaginella 156
3. Isoetes 160

SECTION 10. GYMNOSPERMAE

1. Pinus 164
2. Taxus 173
3. Cycas 177
4. Ginkgo 184
5. Ephedra 187

PART IV.—MORPHOLOGY AND HISTOLOGY OF ANGIOSPERMS

SECTION 1. STEMS AND THEIR MODIFICATIONS

A. External Morphology of Stems

1. The Normal Stem 189

2. Modified Stems

(*a*) Rhizomes 191
(*b*) Tubers 192
(*c*) Corms 193
(*d*) Bulbs 193
(*e*) Stolons 193
(*f*) Runners 194
(*g*) Cladodes 194
(*h*) Thorns 195
(*i*) Other Modifications 195
3. Branching 196
4. Bud Structure 197

B. Primary Histology of Stems

1. Dicotyledons 198
2. Monocotyledons 203
3. Stem Apices 204

PAGE

C. Secondary Histology of Stems
  1. Dicotyledons

    (*a*) Secondary Growth . . . 207
    (*b*) Structure of Mature Wood . . . 210
  2. Monocotyledons . . . 212

SECTION 2. ROOTS AND THEIR MODIFICATIONS
A. External Morphology of Roots

  1. Dicotyledons . . . 213
  2. Monocotyledons . . . 214
  3. Modified Roots . . . 214

B. Histology of Roots
  1. Dicotyledons

    (*a*) Primary State . . . 215
    (*b*) Secondary State . . . 217
  2. Monocotyledons . . . 219

SECTION 3. PERIDERM DEVELOPMENT . . . 221

SECTION 4. LEAVES AND THEIR MODIFICATIONS
A. External Morphology of Leaves
  1. Dicotyledons

    (*a*) Simple Leaves . . . 223
    (*b*) Compound Leaves . . . 224
  2. Monocotyledons . . . 224
  3. Other Types of Foliar Organs . . . 225
  4. Phyllotaxis . . . 226

B. Histology of Leaves

  1. Dicotyledons . . . 226
  2. Monocotyledons . . . 228
  3. Stomata . . . 229
C. Petioles and Leaf Fall . . . 231

SECTION 5. HAIRS AND THEIR MODIFICATIONS . . . 233

SECTION 6. INTERNAL GLANDS

A. Oil Glands . . . 235
B. Latex . . . 236

PART V.—THE SEXUAL REPRODUCTION OF ANGIOSPERMS

SECTION 1. THE MORPHOLOGY AND ANATOMY OF FLOWERS

A. Floral Parts and Symmetry . . . 238
B. Floral Development . . . 240

C. The Structure of the Androecium

  1. Anthers . . . 241
  2. Types of Pollen Grains . . . 242

D. The Structure of the Gynoecium

  1. Ovary and Ovule . . . 243
  2. Development of the Ovule . . . 244
E. Types of Inflorescences . . . 246
  1. Racemose . . . 248
  2. Cymose . . . 248
  3. Bracts and Bracteoles . . . 248

PAGE

SECTION 2. POLLINATION
  A. Entomophily 249
  B. Anemophily 252

SECTION 3. FERTILIZATION 253

SECTION 4. EMBRYO AND SEED
  A. Embryos 254
  B. Seed Structure
    1. Dicotyledons 257
    2. Monocotyledons 259

SECTION 5. GERMINATION
  A. Dicotyledons
    1. Non-endospermic, Hypogeal 260
    2. Non-endospermic, Epigeal 260
    3. Endospermic, Epigeal 261
  B. Monocotyledons
    1. Non-endospermic, Epigeal 261
    2. Endospermic, Hypogeal 261
    3. Endospermic, Epigeal 262

SECTION 6. FRUITS
  A. Dry Fruits
    1. Indehiscent
      (a) Akene 262
      (b) Caryopsis 262
      (c) Cypsela 262
      (d) Samara 263
    2. Dehiscent
      (a) Follicle 263
      (b) Legume 263
      (c) Capsule 263
      (d) Siliqua 264
    3. Schizocarpic
      Schizocarp 264
  B. Fleshy Fruits
    1. Berry 265
    2. Drupe 265
  C. Pseudocarps 266

SECTION 7. DISPERSAL MECHANISMS
  A. Mechanical Dispersal 266
  B. Wind Dispersal
    1. Seeds 267
    2. Fruits 267
  C. Animal Dispersal 268
  D. Water Dispersal 269

PART VI.—THE CLASSIFICATION OF ANGIOSPERMS

PAGE
A SELECTION FROM THE INTERNATIONAL RULES OF NOMENCLATURE . 270

SECTION 1. THE DESCRIPTION OF THE FLOWER
A. Floral Diagrams 270
B. Longitudinal Sections 271
C. Floral Formulae 272

SECTION 2. EXAMPLES OF THE PRINCIPAL FAMILIES
A. Ranunculaceae
1. Ranunculus 272
2. Caltha 273
3. Aconitum 273

B. Papaveraceae
Papaver 273

C. Cruciferae
Cheiranthus 274

D. Violaceae
Viola 275

E. Caryophyllaceae
Stellaria 275

F. Geraniaceae
Geranium 276

G. Papilionaceae
1. Pisum 277
2. Ulex 278

H. Rosaceae
1. Fragaria 278
2. Pyrus 279

J. Saxifragaceae
Saxifraga 279

K. Umbelliferae
Anthriscus 280

L. Compositae
1. Doronicum 280
2. Taraxacum 281

M. Ericaceae
Erica 282

N. Primulaceae
Primula 283

O. Solanaceae
Solanum 283

P. Scrophulariaceae
1. Digitalis 284
2. Veronica 284

PAGE
Q. Labiatae
Lamium 285
R. Betulaceae
Corylus 286
S. Salicaceae
Salix 286
T. Araceae
Arum 287
U. Orchidaceae
Orchis 287
V. Liliaceae
1. Tulipa 288
2. Scilla 289
W. Amaryllidaceae
Narcissus 289
X. Gramineae
Arrhenatherum 290

PART VII.—PLANT PHYSIOLOGY

HYDROGEN ION CONCENTRATION: THE MEASUREMENT OF *p*H . . . 291

SECTION 1. COLLOIDAL PHENOMENA

A. Lyophile Sols 292
B. Emulsions and Lyophobe Sols 292
C. Flocculation of Colloid Sols 294
D. Brownian Movement 295
E. Effect of Reaction (*p*H): Amphoteric Colloids . . 295
F. Imbibition 296
G. Adsorption 298
H. Surface Tension 300
J. Dialysis 302

SECTION 2. ABSORPTION

A. Osmosis through a Semi-permeable Membrane . . 302
B. Artificial Osmotic Cells 304
C. Potato Osmoscope 304
D. Osmotic Potential of a Solution 305
E. Osmotic Pressure in Living Cells 306
F. Suction Force 306
G. Tissue Strains 308
H. Plasmolysis 308
J. Penetration by Solutes 309
K. Changes in Permeability 310
L. Water Cultures 311
M. Absorption of Water by the Seed 313

PAGE

SECTION 3. TRANSPIRATION

A. The Potometer 313
B. Comparison of Absorption and Transpiration . . . 316
C. The Cobalt Chloride Test for Transpiration . . . 318
D. Measurement of the Evaporating Power of Air . . 319
E. Comparison of Stomatal and Cuticular Transpiration . 319
F. The Porometer 321
G. Movement of Water in a Shoot 322
H. Permeability of Wood to Water 323
J. Root Pressure 325
K. Askenasy's Experiment 326

SECTION 4. ASSIMILATION

A. Formation of Starch in the Leaf 327
B. Assimilation of Carbon Dioxide from the Air . . . 330
C. Evolution of Oxygen in Photosynthesis 330
D. Gas Exchange in Photosynthesis 335
E. Dry Weight Method of Measuring Photosynthesis . . 337
F. Extraction of Chlorophyll : Absorption Spectrum . . 337
G. Separation of the Leaf Pigments 339
H. Etiolation 341
J. Nitrogen Assimilation : Presence of Nitrates and Nitrites in Tissues 341
K. Translocation 342

SECTION 5. ENZYMES

A. Action of Amylase on Starch 342
B. Action of Lipase on Fat 344
C. Action of Protease on Proteins 344
D. Oxidizing Enzymes 345

SECTION 6. RESPIRATION

A. Aerobic Respiration 347
B. Respiration of Starchy and Fatty Seeds 348
C. Quantitative Measurement of Respiration . . . 351
D. Excretion of Carbon Dioxide by Roots ; Microscopic Observations 353
E. Production of Heat by Respiration 354
F. Loss of Weight due to Respiration 356
G. Anaerobic Respiration 356
H. Fermentation of Sugar by Yeast 357

SECTION 7. GROWTH

A. Germination of Seeds 359
B. Distribution of Growth in Root, Stem and Leaf . . 360
C. Measurement of Growth with the Auxanometer . . 361
D. Root Auxanometer 363
E. Effect of Temperature on Growth 364
F. Circumnutation 366
G. Growth Pressure 367
H. Effect of Growth Substances 368

PAGE

SECTION 8. RESPONSE
- *A*. Experiments on Geotropism 369
- *B*. Klinostat 370
- *C*. Geotropism in Relation to Respiration 371
- *D*. Phototropic Response 372
- *E*. Phototropism with Light of Different Colours and Intensities 373
- *F*. Nyctinastism of Leaves 374
- *G*. Hydrotropism of Roots 374
- *H*. Chemotropism and Chemotaxis 376
- *J*. Contact Sensitiveness (Thigmotropism) 378
- *K*. Shock-Sensitiveness 378
- *L*. Hygroscopic Movements 379
- *M*. Cyclosis 381

PART VIII.—MODIFICATIONS OF THE NORMAL PLANT FORM

SECTION 1. ANATOMICAL MODIFICATIONS
- *A*. Leaves
  - 1. Xerophytes 382
  - 2. Aquatics 385
  - 3. Sun and Shade Leaves 386
- *B*. Stems
  - 1. Xerophytes 386
  - 2. Aquatics 387
  - 3. Halophytes 389
  - 4. Etiolation 390
- *C*. Roots 390

SECTION 2. CLIMBERS
- *A*. Twiners
  - 1. External Morphology 391
  - 2. Anatomy 392
- *B*. Hook Climbers 393
- *C*. Tendril Climbers 394

SECTION 3. EPIPHYTES 395

SECTION 4. PARASITES
- *A*. Stem Types 396
- *B*. Root Types 398
- *C*. Algal Types 399

SECTION 5. SYMBIOSIS AND SPECIAL MODES OF LIFE
- *A*. Lichens 400
- *B*. Mycorrhiza 402
- *C*. Root Nodules 402
- *D*. Carnivorous Plants
  - 1. Utricularia 403
  - 2. Nepenthes 404
  - 3. Pinguicula 406
  - 4. Drosera 406
  - 5. Dionaea 408

PAGE

SECTION 6. VEGETATIVE PROPAGATION
A. Natural Propagation
  1. Stolons 408
  2. Suckers 409
  3. Offsets 409
  4. Bulbils 409
  5. Layers 411
  6. Hibernacula 411
  7. Adventitious Buds 412
B. Artificial Propagation
  1. Stem Cuttings 413
  2. Root Cuttings 413
  3. Callus Formation 414

PART IX.—PLANT ECOLOGY

INTRODUCTORY REMARKS ON FIELD WORK 415

SECTION 1. SOIL STUDIES
A. Analysis of Soils
  1. Sampling 416
  2. Mechanical Analysis 417
  3. Microscopical Examination 418
B. Physical Properties
  1. Natural Moisture Content 418
  2. Saturation Capacity 419
  3. Pore Space 419
  4. Moisture Equivalent 420
  5. Capillary Power 420
  6. Sticky Point 420
  7. Flocculation of Clay 421
  8. Percolation through Soil 422
C. Chemical Properties
  1. Soil Reaction 422
  2. Carbonate Content 422
  3. Lime Deficiency 424
  4. Lime Status and Lime Requirement . . . 424
  5. Exchangeable Calcium 425
  6. Total Exchangeable Bases 426
  7. Nitrates in Soil 426
  8. Total Organic Content 426
  9. Oxidizable Organic Content 427

SECTION 2. PHYSIOLOGICAL OBSERVATIONS
A. Transpiration 427
B. Atmometry 429
C. Illumination
  1. Leaf Mosaic 431
  2. Etiolation 433

SECTION 3. HERBARIUM TECHNIQUE 433

PART X.—VARIATION AND HEREDITY

PAGE

SECTION 1. VARIATION
  A. Fluctuating or Chance Variation
    1. Construction of the Curve of Chance 437
    2. Natural Examples of Chance Variation . . . 437
  B. Correlated Variation 439

SECTION 2. MUTATIONS 441

SECTION 3. EVIDENCES OF EVOLUTION
  A. Ontogenetic Evidence 445
  B. Geographical Evidence 445

SECTION 4. HYBRIDIZATION AND INHERITANCE
  A. Experimental Hybrids 446
  B. Natural Hybrids 448
  C. Variation and Hybridization within Natural Species . 448
  D. Graft Hybrids 451

APPENDIX

RECEIPTS FOR COMMON STAINS AND REAGENTS USED IN THIS BOOK

SECTION 1. MICROSCOPICAL REAGENTS
  A. Fixatives 454
  B. Macerating Fluids 455
  C. Stains 456
  D. Mounting Media 458

SECTION 2. CHEMICAL REAGENTS
  A. Microchemical Test Reagents 458
  B. Indicators 460
  C. Water Culture Solutions 461
  D. Agar Media 461

SECTION 3. REFERENCE TABLES 462

SECTION 4. ADDRESSES OF SCIENTIFIC SUPPLIERS IN GREAT BRITAIN
  A. General Apparatus, Glassware, etc. 465
  B. Chemicals 465
  C. Stains and Indicators 465
  D. Microscopes, etc. 465
  E. Botanical Material 465

FOUR-FIGURE LOGARITHMS 466

ANTILOGARITHMS 468

INDEX OF PLANT MATERIAL REFERRED TO IN THIS BOOK 471

INDEX OF SELECTED SUBJECTS 475

LIST OF ILLUSTRATIONS

PAGE

1. A students' microscope 5
2. Method of cutting a hand section 13
3. A good form of hand microtome 15
4. Stages of mitosis in the root of *Vicia faba* 37-38
5. *Pandorina morum.* A typical colony 56
6. *Eudorina elegans.* Surface and sectional views of colony 57
7. *Eudorina illinoisensis.* Formation of autocolonies 57
8. *Oedogonium crassiusculum.* Oogonium with nannandrous male plant . 64
9. *Polysiphonia nigrescens.* Cystocarp in surface and sectional views . . 69
10. *Achlya colorata.* Oogonium with associated antheridia 87
11. *Pythium debaryanum.* Oogonium with antheridium 89
12. *Cystopus candidus.* Mature oospore 91
13. *Erysiphe cichoracearum.* Vertical section through perithecium . . 94
14. *Saccharomyces cerevisiae.* Sexual reproduction 97
15. *Puccinia graminis.* Aecidia and pycnidia on Barberry 100
16. *Puccinia graminis.* Uredosorus on Wheat 100
17. *Puccinia graminis.* Teleutosorus on Wheat 101
18. *Psalliota campestris.* Vertical section through young sporophore . . 103
19. *Bacillus sporogenes.* Individuals with endospores 107
20. *Marchantia polymorpha.* Horizontal section through the archegoniophore 115
21. *Mnium hornum.* Longitudinal section through apex of female plant . 119
22. *Mnium hornum.* Longitudinal section through capsule 121
23. *Mnium hornum.* Structure of the peristome 122
24. *Polytrichum commune.* Transverse section of aerial stem 126
25. *Polytrichum commune.* Transverse section of rhizome 127
26. *Polytrichum commune.* Structure of the peristome 129
27. *Dryopteris filix-mas.* A dissected stele 133
28. *Dryopteris filix-mas.* Vertical section of the antheridium 138
29. *Dryopteris filix-mas.* Vertical section of the archegonium 138
30. *Equisetum arvense.* Prothallus with archegonia 153
31. *Equisetum arvense.* Prothallus with antheridia 154
32. *Lycopodium clavatum.* Longitudinal section of several sporophylls . . 156
33. *Selaginella kraussiana.* Longitudinal section of the strobilus . . . 158
34. *Selaginella kraussiana.* Section through megaspore to show female prothallus 159
35. *Pinus sylvestris.* Longitudinal section of male cone 169
36. *Pinus sylvestris.* Vertical section of the ovule showing archegonia . . 172
37. *Taxus baccata.* Longitudinal section of male cone 176
38. *Taxus baccata.* Longitudinal section through the ovule 177
39. *Cycas revoluta.* Habit of plant 178
40. *Zamia floridana.* Transverse section of the stem 178
41. *Macrozamia miquelii.* Male cone 181
42. *Cycas revoluta.* Strobilus of megasporophylls 182
43. *Cycas revoluta.* Single megasporophyll 183
44. *Stangeria paradoxa.* Vertical section of the young ovule in an early stage of the development of the female gametophyte 184
45. *Vicia faba.* Course of vascular bundles at the node 203

PAGE
46. *Tropaeolum majus.* Longitudinal section of stem apex 205
47. *Hippuris vulgaris.* Longitudinal section of stem apex 206
48. *Laburnum anagyroides.* Transverse section of wood block 211
49. *Dracaena* sp. Transverse section of the stem to show secondary thickening 212
50. *Vanda teres.* Transverse section of an aerial root 220
51. *Zea mais.* Superficial section to show stomata 231
52. *Drosera rotundifolia.* Vertical section of a leaf with gland 234
53. *Euphorbia splendens.* Longitudinal section of the stem with latex tubes . 237
54. *Helianthus rigidus.* Longitudinal section of the young capitulum . . 240
55. *Lilium* sp. Sections of mature pollen grains showing the nuclei . . 242
56. *Narcissus majalis.* Longitudinal section of the ovule and embryo sac . 245
57. *Lilium* sp. Stages in meiosis in the embryo sac 247
58. *Eschscholtzia californica.* Pollen developing on the stigma 253
59. *Capsella bursa-pastoris.* Stages in the development of the embryo . . 256
60. *Zea mais.* Longitudinal section through the seed 259
61. Diagram of the arrangement of the comparison tubes in *p*H determination 292
62. Apparatus to demonstrate the Tyndall effect 293
63. Apparatus to demonstrate the swelling power of seeds 297
64. Apparatus to measure surface tension 301
65. Apparatus to demonstrate osmotic pressure 303
66. A series of water culture experiments 312
67. The Darwin potometer 314
68. The Thoday potometer 316
69. Apparatus to compare absorption and transpiration 317
70. Method of determining transpiration using Cobalt chloride paper . . 318
71. Apparatus to demonstrate cuticular transpiration 320
72. The porometer 321
73. Apparatus to compare the relative permeability of woods 324
74. Apparatus to measure root pressure 325
75. Zonal Pelargonium (Geranium) leaves after screening 328
76. Graphs of the relative absorptions of coloured screens 328
77. A plant with coloured screens attached 329
78. Apparatus to demonstrate the evolution of Oxygen by *Elodea* . . . 331
79. Apparatus to measure the Oxygen evolved by *Elodea* 332
80. The Indigo-carmine method of demonstrating the evolution of Oxygen by the leaves of water plants 334
81. Apparatus to illuminate plants during photosynthesis experiments . . 334
82. The Ganong photosynthometer 336
83. The Tswett apparatus 340
84. Chromatogram obtained with the Tswett apparatus 340
85. The McDougall respiroscope for the comparison or respiration in starchy and fatty seeds 349
86. The Ganong respirometer 350
87. Gas-train to measure the Carbon dioxide evolved by seeds during respiration 351
88. Thoday's pattern of simplified Barcroft apparatus 353
89. Apparatus to demonstrate the evolution of Carbon dioxide by roots . 354
90. Apparatus to demonstrate the heat of respiration in seeds 355
91. Apparatus to demonstrate anaerobic respiration 357
92. Apparatus to demonstrate the fermentation of yeast 358
93. Method of marking Bean roots to demonstrate the region of active growth 360
94. A simple lever-pattern auxanometer 362
95. Farmer's drum-pattern auxanometer 362
96. The Neilson Jones auxanometer for roots 364

PAGE
97. Measurement of root growth by a measuring microscope 365
98. Apparatus to map the circumnutation of a shoot 367
99. Apparatus to demonstrate growth pressure 368
100. Two klinostats (Small's). That on the left is rotating while that on the right is stationary 371
101. Method of demonstrating the action of light on seedlings . . . 372
102. Nyctinastic movement in the leaves of *Oxalis corniculata* . . . 375
103. Demonstration of hydrotropism in roots 376
104. Comparison of a plant of *Mimosa pudica* before and after shock . . 379
105. Apparatus to demonstrate the hygroscopic movement of the awn of *Stipa tenacissima* 380
106. *Hakea pectinata.* Transverse section of the leaf 383
107. *Cuscuta epithymum*, on *Trifolium*. Section through host to show invasion 396
108. *Viscum album.* Section through host to show sinkers 397
109. *Polysiphonia fastigiata.* Section to show the invasion of the Brown Algal tissue by the Red Alga 399
110. *Xanthoria parietina.* Vertical section through the edge of an apothecium 402
111. *Utricularia vulgaris.* Vertical section through the trap . . . 404
112. *Nepenthes* sp. A single leaf to show the parts 405
113. *Nepenthes* sp. Vertical section through a pitcher gland . . . 405
114. *Dionaea muscipula.* A plant showing the modified leaves . . . 407
115. *Dionaea muscipula.* Vertical section through the leaf 407
116. *Festuca vivipara.* Flower head showing the young plants developing in place of flowers 410
117. *Bryophyllum tubiflorum.* Young plants growing from the tips of the leaves 413
118. Apparatus for determining the Calcium carbonate content of soils . 423
119. The Casella atmometer 430
120. *Aesculus hippocastanum.* Young plant showing a leaf mosaic . . 432
121. *Cotoneaster horizontalis.* A shoot showing the leaf mosaic . . . 432
122. *Taxus baccata.* A, normal type. B, mutant type 441
123. *Fagus sylvatica.* A, normal foliage. B, mutant cut-leaved type . . 442
124. *Chelidonium majus.* A, normal type. B, laciniate mutant . . . 444
125. *Viola.* Natural hybrid between *V. lactea* and *V. riviniana* . . . 449
126. *Plantago coronopus.* Variation in leaf form 451
127. *Laburnum adami.* A graft hybrid of *Laburnum anagyroides* and *Cytisus purpureus* 452

OUTLINE CLASSIFICATION OF PLANTS

Thallophyta.—Plants with no distinction of stem or leaf. Plants usually simple, sometimes unicellular.

Algae.—Thallophytes with chlorophyll, sometimes with additional pigments which conceal the chlorophyll.

Chlorophyceae (Green).—Freshwater or marine, many unicellular.

Chlamydomonas.
Haematococcus.
Pandorina.
Eudorina.
Volvox.
Ulothrix.
Pleurococcus.
Spirogyra.
Vaucheria.
Oedogonium.

Cyanophyceae (Blue green).—Chiefly freshwater. Blue colour due to phycocyanin. No sexual reproduction.

Nostoc.

Rhodophyceae (Red).—Chiefly marine. Red colour due to phycoerythrin.

Batrachospermum.
Chondrus.
Polysiphonia.
Corallina.

Phaeophyceae (Brown).—Marine. Brown colour due to fucoxanthin.

Ectocarpus.
Cutleria.
Dictyota.
Laminaria.
Fucus.

Fungi.—Thallophytes with no chlorophyll, living either as saprophytes or parasites.

Archimycetes.—Thallus amoeboid, devoid of hyphae. Reproduction by asexual spores or motile isogametes.

Plasmodiophora.
Fuligo.
Arcyria.
Reticularia.

Phycomycetes.—Hyphae non-septate or absent. Reproduction partly by asexual spores, partly by sexually produced zygotes.

Mucor.
Saprolegnia.
Monoblepharis.
Pythium.
Cystopus.
Peronospora.

Ascomycetes.—Hyphae septate. Fruiting bodies producing ascospores inside sac-like asci.

Aspergillus.
Erysiphe.
Peziza.
Rhytisma.
Saccharomyces.
Claviceps.
Hypoxylon.

Basidiomycetes.—Hyphae septate. Fruiting bodies developing basidiospores on basidia.

Puccinia.
Polyporus.
Psalliota.
Cyathus.
Phallus.

Bacteria.—Thallophytes with no chlorophyll. Unicellular. Nutrition variable.

Eubacteriales.—Minute organisms, spherical or rod-like, reproducing only by fission or by non-sexual spores.

Bacillus.
Staphylococcus.

Charophyta.—Stems jointed, with whorled branches. Joints mostly consisting of single cells.

Chara.

Bryophyta.—Plants with no true roots and no woody tissue. Sporophyte dependent on gametophyte. Gametophyte leafy or thalloid. Sporophyte bearing spores in leafless capsules.

Hepaticae.—Gametophyte thalloid or with stems bearing leaves. Sex organs terminal or dorsal. Sporophyte consisting of non-chlorophyllous sporogonia.

Pellia.
Marchantia.
Anthoceros.

Musci.—Gametophyte of tufted stems bearing leaves. Sex organs terminal. Sporophyte leafless, consisting of chlorophyllous sporogonia.

Mnium.
Funaria.
Polytrichum.
Sphagnum.

Pteridophyta.—Plants containing woody tissue and with true roots, but with no flowers. Sporophyte independent of the gametophyte.

Filicales (Ferns).—Sporangia in clusters on the leaves.

Dryopteris.
Pteridium.
Hymenophyllum.
Osmunda.
Botrychium.
Azolla.

Equisetales (Horsetails).—Leaves merely scales; sporangia in leafless terminal strobili.

Equisetum.

Lycopodiales (Club Mosses).—Sporangia borne singly in the axils of leaves, usually aggregated into strobili.

Lycopodium.
Selaginella.
Isoetes.

Spermatophyta.—Plants forming true seeds.

Gymnospermae.—Plants with seeds attached to woody scales, not enclosed in a fruit. Scales usually forming a cone.

Coniferales. Flowers in the form of woody cones. Leaves reduced to needles or scales.

Pinus.

Taxales. Flowers in very reduced cones, comprising a few microsporangia or ovules only.

Taxus.

Cycadales. Flowers in the form of large woody cones. Leaves large and pinnate.

Cycas.

Ginkgoales.—Flowers, male, in simple cones, female terminal on stalks. Leaves fan-shaped.

Ginkgo.

Gnetales.—Flowers with perianth, in reduced cones. Leaves undivided. Xylem vessels present.

Ephedra.

Angiospermae.—Plants with seeds enclosed in a fruit. Flowers usually with coloured petals.

Dicotyledons.—Seedlings with two cotyledons; leaves net-veined.

Archichlamydeae (Polypetalae).—Flowers with petals not united.

Ranunculus.
Caltha.
Aconitum.
Papaver.
Cheiranthus.
Viola.
Stellaria.
Geranium.
Pisum.
Ulex.
Fragaria.
Pyrus.
Saxifraga.
Anthriscus.
Corylus.
Salix.

Metachlamydeae (Sympetalae).—Flowers with petals united.

Taraxacum.
Doronicum.
Erica.
Primula.
Solanum.
Digitalis.
Veronica.
Lamium.

Monocotyledons.—Seedlings with one cotyledon; mature leaves mostly parallel-veined.

Arum.
Orchis.
Scilla.
Tulipa.
Narcissus.
Arrhenatherum.

PART ONE

METHODS

Section 1. Objects and Methods of Laboratory Study

A. HISTORY

Laboratory studies in Botany are of comparatively recent growth in the history of Science. About a century ago a University course in Botany was seldom taken by any but medical students, and the type of course then prevalent was well exemplified by that given by Professor Daniel Oliver at University College, London. The class met in the old Botanical Theatre, a semicircle of benches in tiers, facing a lecture table. Before each class the Professor himself went round and laid out on each place half a dozen fresh flowers, newly delivered from Kew Gardens. The students arrived, the Professor stepped up to his table, and an hour was spent in the detailed description and examination of the specimens provided. That was all. Botany was a purely descriptive and classificatory subject.

Of course, anatomical and physiological studies had begun long before this, but they were confined to a few specialists, and no one attempted to introduce students or school pupils to such work.

An advance towards a wider form of instruction began in the German Universities, especially at Bonn and Tübingen, in or about the year 1870, and the introduction of the new ideas to Great Britain soon followed, fostered particularly by the enthusiasm of Thistleton Dyer and F. O. Bower. This development was only rendered possible by the production in Germany of a simple form of students' microscope, more suitable both in its simplicity and its price than the elaborate instruments turned out by British makers for the wealthy amateur. Thus arose the tradition of the superiority of German microscopes for scientific work which still lingers in some quarters, though happily one may now be allowed to stigmatize it as a baseless legend.

The microscope became, however, and has remained, the biologist's primary instrument, and a large part of the present book is concerned with microscopic observations. The first microscope demonstrations were given under Huxley's guidance in the "Science and Art" classes at South Kensington in 1873, and Thistleton Dyer recorded the astonishment and pleasure which the participants experienced (teachers as well as students, for all were fellow-adventurers) in finding themselves easily able to observe objects which had hitherto been seen only by the erudite researcher. That pure delight which flows from personal contact with the withdrawn and secret aspects of nature can be captured anew by any student gifted with a little patience and a little imagination, if he has a guide. It is the object of this book to make his way easier for him. For the pupil in a class it will supplement the demonstrations of the teacher, while it should also make it possible for the amateur naturalist to find his own way through studies of a kind generally regarded as needing expert tuition.

B. THE SCOPE OF LABORATORY STUDIES

Botany is essentially a practical subject and ideally the study of plants should be carried out in the field, for it is highly desirable that the plant should be regarded as a living organism, living in and affected by its environment, which profoundly affects its morphology and often its anatomy as well. Laboratory work must therefore be regarded as an adjunct to field work, not a substitute. For this reason the material used in laboratory work should, if possible, be collected by the student himself. Pieces of material preserved in alcohol can give little idea of the appearance of the living plant or even of its morphology, and a student cannot be expected to gain a proper appreciation of plants simply from a study of preserved material.

It is for this reason that the types selected for study have, as far as possible, been chosen from those which occur wild in Great Britain. In many cases, in addition to the main types described, alternatives, which may be more readily obtainable locally, have been suggested. On the other hand, it must be borne in mind that there are many further suitable plants which will illustrate the point under consideration, and the student should be encouraged to examine other material and see for himself whether it is suitable.

Many University Departments have Botanic Gardens or, alternatively, have a working arrangement with the public parks whereby students can obtain material, and they should be encouraged to make the fullest use of them. Students of Botany must realize that the best time to study plants is in the summer, and the fact that this is largely a period of vacation must not be allowed to interfere with the study of Botany even if the laboratories are closed. Indeed, much field work can only be successfully carried out during the summer months.

The present book is intended to deal exclusively with the laboratory side of botanical study, for the field work has already been treated in the authors' book, " Practical Field Ecology." All the descriptions of the material used in this book are based upon average specimens, but the student must expect some variation in living organisms. It should also be borne in mind that, though frequently the account given draws attention to a particular feature, the same material, or even the same section, can be used to illustrate other features as well, so that in many cases the study of a particular specimen can be greatly extended.

The order of treatment is intended to be logical rather than chronological. The exigencies of the seasons do not make the strictly logical order possible in practice. Moreover, while the purely logical order is something more or less fixed and accepted, no two teachers will agree as to the best practical sequence, for the good reason that every teacher knows his own circumstances best. The book is therefore in no sense a syllabus.

C. PRINCIPAL METHODS

The principal methods of microscopical study in Botany are :—

Dissection.—This is only of minor importance in Botany and applies chiefly to flowers. Microdissection or " teasing out " under the microscope, with fine needles, is often needed with minute structures.

Section Cutting.—The thinnest possible slices, cut with a razor, are laid on a glass slip in a drop of liquid and covered with a thin glass cover for microscopic examination. This is now the universal technique for studies in plant anatomy.

Drawing.—This, properly speaking, embraces both the above, for it should be the student's golden rule that *nothing has been truly observed unless it has been drawn.* This is literally true, but only experience will prove how true it is. Drawing forces relationships of structure on our notice in a way that nothing else can. Moreover, every drawing of an object is an interpretation of it, and unless one understands a structure one cannot make a good drawing of it. Drawing is therefore the best aid to understanding. It requires time, of course, and labour, but so do nearly all things that are worth doing.

Experiment.—Botany has become, to a large extent, an experimental science, and the predominance of the experimental method increases as knowledge advances. Research methods are largely experimental and quantitative. Do not get the idea that Botany is an inexact science in comparison with Physics and Chemistry. It is not. The working of a living organism is as precise as that of a machine, and the whole aim of our study should be by accuracy of observation to match the accuracy of the organism. We cannot be too accurate for our material.

This demand for accuracy is part of the intellectual value of botanical study, which is quite distinct from any economic value that Botany may have. It is an excellent and varied discipline ; but, if accuracy be sacrificed to convenience, if slipshod methods and half-comprehensions are accepted and tolerated, all that value is lost and the sense of value itself is blunted, as it always is by bad work consciously indulged.

D. THE NECESSARY MATERIALS

A few words are required here about the instruments for the work. Send to a laboratory furnisher and get the proper things. It is worth while. A full set of dissecting instruments is not needed. Instead, purchase the following separately :—

1 Razor, flat-sided for section cutting. Hollow-ground razors are not good for this purpose, nor are safety razor blades.

1 Razor strop.
1 Scalpel, or sharp penknife.
2 Needles, mounted in wooden handles.
1 pair small forceps.
1 Medium-sized camel-hair brush.
3 Watch-glasses or similar small shallow dishes.
½ Gross box of microscope slides, 3 in. by 1 in.
½ oz. of microscope cover slips, ¾ circles, No. 2 thickness.
½ dozen small dropping-bottles for reagents. The simplest kind are little narrow-necked bottles, with a short length of glass rod passing through the cork into the bottle. The corks should be waxed.
A *clean* handkerchief for polishing slides and cover slips.
A supply of good, hard-surfaced drawing paper, size about 11 in. by 10 in.
A folder-cover of some kind to hold the completed drawings. This is preferable to buying a bound drawing book, since it allows your drawings to be arranged in correct grouping no matter in what order they are done.
A *hard* pointed pencil.
A white glazed tile about 6 in. square will be found very useful for working on and it is best to enamel half of it in black.
A slide-box for preserving permanent microscopical preparations.

Lastly, there is the question of a pocket lens. There is frequent need for it in the laboratory, and it is indispensable to the naturalist out of doors. If you have one you will quickly find innumerable uses for it and learn to cherish it as you cherish your pipe or your powder compact, so by all means get a good one. The aplanatic types are the best, though expensive, for they give a flat field of view, but in any case get one magnifying at least ten diameters.

Section 2. The Microscope

A. PURCHASING A MICROSCOPE

Many firms make **students' microscopes** and it is not easy to make a choice between the models on the market, but fortunately the student will not go far wrong if he sticks to one of the makers of repute. The principal makers of microscopes are listed in the Appendix (see p. 465).

Any instrument bought from one of these firms should give perfect satisfaction, and the purchaser may safely let himself be guided by price and personal taste. Do not buy a second-hand instrument without some expert advice other than that proffered by the vendor, and *on no account* buy any microscope which does not bear a maker's name, however cheap it is. The older makers, such as Powell and Lealand, Crouch or Ross, whose names you will often find on old instruments, made good microscopes in their time, but they are mostly out of date now.

In making a choice you must, however, distinguish between *essentials* and *accessories*, for the latter make up a high proportion of the cost of the more expensive instruments.

B. ESSENTIALS OF THE MICROSCOPE

The foundation of the instrument is a **foot** with a **limb** (Fig. 1) rising from the foot. The limb bears the **stage,** on which objects are placed and fixed by **stage-clips.** Below the stage is the **mirror,** to illuminate the objects. Above is the **body,** a brass tube which is moved vertically by a rack and pinion called the **coarse adjustment.** These constitute the essentials of the **stand.** Magnification is not a function of the microscope stand as such, but of the interchangeable lenses which you attach to it. The mechanical stand is only to enable these lenses to be properly used. Thus, for example, if high powers are used a **fine adjustment** to the body becomes an additional essential, but it is not essential with low powers. The body is often furnished with an inner **draw-tube,** but this is not actually an essential.

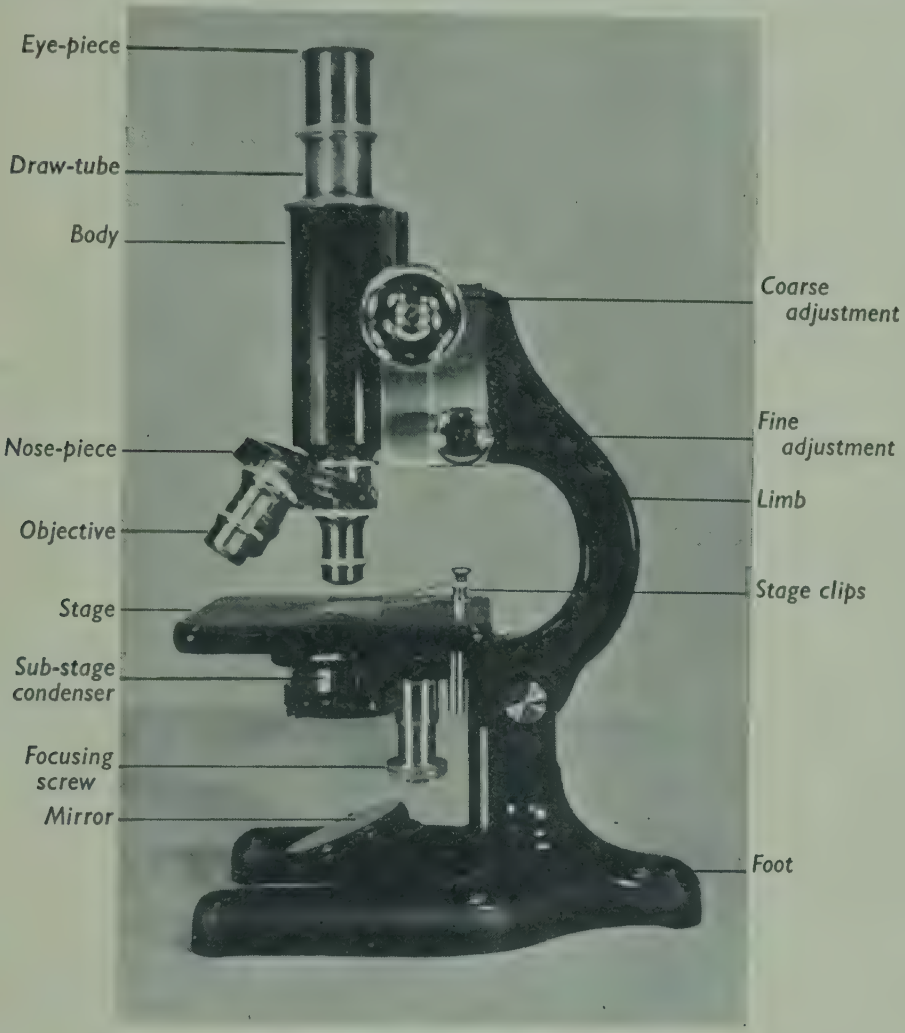
Eye-piece
Draw-tube
Body
Coarse adjustment
Fine adjustment
Limb
Nose-piece
Objective
Stage
Stage clips
Sub-stage condenser
Focusing screw
Mirror
Foot

FIG. 1.—A student's microscope.

The lenses (or rather the lens systems) are two. At the top of the body you insert an **eye-piece** (or **ocular**) and at the lower end you screw on an **objective.** The latter forms an enlarged image of the object, the former magnifies this image further. The total magnification is therefore the product of the magnifying power of both lenses and may be varied by changing either or both of them.

Do not seek for extreme magnification. It brings many disadvantages. One eye-piece, magnifying eight or ten times, is enough for general use, and there should be at least two objectives, one the "low power" of 16 mm. ($\frac{2}{3}$ in.) focal length, and one of 4 mm. ($\frac{1}{6}$ in.), the "high power." The use of these is assumed in this book.

C. ACCESSORIES

To the above equipment you may add at pleasure: (1) A **sub-stage condenser,** *i.e.*, a lens below the stage for concentrating light on the object. It is very valuable with the high power if properly used. It also carries an **iris diaphragm** for controlling the light and, usually, a holder for light filters. (2) A **nose-piece**: a device for holding and rapidly changing the objectives. This is practically an essential. (3) A **mechanical stage,** which enables you to move objects with greater precision. (4) An **oil-immersion lens**: 2 mm. ($\frac{1}{12}$ in.), which is used with a drop of thickened cedar wood oil between the lens and the cover-glass. This gives roughly twice the magnification of the 4 mm. lens and greater resolution. It is useful for very fine details and is really essential for bacteria.

The above are the most valuable accessories, but the catalogues are full of "gadgets," if you have a taste for them.

The school teacher is often faced with the problem of making one microscope do for a whole class. In such a case one may recommend a small **microprojector** rather than an ordinary microscope. This has a powerful lamp which throws the enlarged image on to a screen, like a lantern slide. If a small screen is used near the instrument, the image is bright enough to see in daylight, and a small class can all view it at once. Drawings can also be made by tracing the projected image.

D. USING THE MICROSCOPE

The astronomer carefully studies his telescope, the chemist understands his balance, but the biology student is supposed by many teachers of the "Shove-it-Under" school of microscopy to know all he needs to know about the microscope by the light of nature. The ignorance of teachers themselves is too often responsible for this neglect, and the "instructions" sometimes given are enough to make the good microscopist sigh.

There is only one correct way to use a microscope. Get it firmly fixed in your mind by practice and never depart from it. It is not difficult and you will be amply repaid by results.

Get a good light, either a north light in daylight (*never* direct sunlight if you value your eyes!) or an opal or pearl electric bulb.

See that your lenses are clean. If not, polish them very, very gently with a chamois leather or a soft handkerchief. Never use a scratchy duster, and keep your fingers off the lenses at all times.

Drop the eye-piece into the body and screw the objectives on to the nose-piece. Place the **low power** in the working position under the body.

Turn the microscope with the limb towards you. Turn up the **concave** side of the mirror, look through the eye-piece and turn the mirror about until you find the best light.

Place the object on the stage and focus the **low power** on it (about ½ in. from the cover glass) with the milled heads of the coarse adjustment. Always make a survey of the object with the low power first.

If you have a **sub-stage condenser,** turn up the **flat** side of the mirror and then focus the condenser, with the movement provided, so that the image of the lamp or, in daylight, of some distant object, appears clearly defined in the field of view. This is the correct position for the condenser to give " critical illumination " and it must be adhered to. If, under the low power, you find the image of the lamp, etc., in the field of view distracting, insert a piece of ground glass between the mirror and the light, but do not alter the setting of the condenser.

When you have found something in the object that needs further magnification, raise the objective a little and turn the high power into position. Then, watching this *from the side*, lower it carefully until it is practically touching the cover glass. Look through the eye-piece and *raise* the objective with the coarse adjustment till the object comes into focus (about 1 mm. from the cover glass). Complete the focusing with the fine adjustment only.

Practise using both eyes alternately and practise keeping open the eye not in use. This saves a great deal of strain, and however difficult it may seem at first it will rapidly become an unconscious habit.

Dark-ground illumination is not often needed in botanical work, though it is sometimes very useful as well as beautiful. With low powers it is easily obtained by attaching a small disc of black paper to the lower side of the sub-stage condenser, so as to stop out all the central rays of light, leaving only the peripheral ones. These emerge from the top of the condenser so obliquely that they do not enter the objective. The general field is therefore dark, but any object at the focus of these oblique rays scatters light by reflection and appears as if self-luminous. Coloured light may be used to add to the spectacular effect.

With high powers the operation is more difficult and special wide-angle condensers are needed. This class of illumination is mostly of use for living Bacteria and Protozoa and is outside the scope of this book.

E. MEASUREMENT

It is excellent practice to start microscopic measurement from the beginning. A finely engraved scale is placed in the eye-piece. The value of the divisions of this scale will vary with the magnification used, so that they must be calibrated for each objective by comparison with a ruled **stage micrometer,** which is a glass slide bearing a fine scale, usually of tenths and hundredths of a millimetre. Both types of scale are sold by the microscope makers.

A simpler though somewhat less accurate procedure is to project the microscopical image, using a strong light, on to a piece of paper at a distance of 10 in. from the eye-piece. Measure this image and divide by the magnification used. This will be, for a 16 mm. objective and a × 10 eye-piece, about 100 diameters, and for a 4 mm. objective and the same eye-piece, about 450 diameters. Reference to the table below will give you the exact figures for common objectives.

Table of Magnifications

Makers.	Achromatic Objectives.		
	16 mm. (2/3)	4 mm. (1/6)	2 mm. (1/12)
C. Baker	10·0	40·0	100·0
Bausch & Lomb	10·0	43·0	97·0
R. & J. Beck	11·5	38·5	100·0
Cooke, Troughton & Simms	12·0	50·0	119·0
W. R. Prior	10·0	42·0	95·0
Spencer Lens Co.	10·0	44·0	95·0
J. Swift	8·5	41·0	92·5
W. Watson	9·0	42·0	84·0
Leitz	10·0	45·0	100·0
Reichert	10·0	45·0	100·0
Zeiss	10·0	40·0	90·0

The above magnifications are the basic magnifications given by the specified objective at a tube length of 160 mm. The actual magnification is the basic magnification multiplied by the magnification of the eye-piece employed.

F. DRAWING

When drawing a preparation place the paper close to the right-hand side of the microscope. If you then use your left eye for observation you will soon find it possible to transfer what you see with the left eye to the paper which you see with your right eye. Some people find this easy, others very difficult. Try it for yourself, as it makes things much simpler for you if you can do it.

There is, however, an instrument called a **camera lucida** which will do it for you by means of a reflecting prism and a mirror. The price of a good one lies between £10 and £15 and you are not likely to need it for elementary studies, but for the research worker, or when true-to-scale drawings are required, it is virtually a necessity.

Drawings with the microscope are usually one of two kinds: either **diagrams,** in which the general arrangement of the tissues in a section is represented schematically by means of outlines and the tissues distinguished merely by means of different hatchings ; or **drawings,** properly so called, in which the details of cell construction are represented as accurately as possible. The former are more suitable for low-power views, the latter for high-power examination. Whichever is attempted, be clear what you are doing and do not perpetrate a vague mixture of both styles. Never attempt to represent the cells in a diagram and never be schematic in a drawing.

When making a drawing lay down the shapes of the cells first of all with single lines, representing middle lamellae, then build the thickness of the various cell walls on these separately, finishing off carefully with thin, precise lines, with a hard pencil or drawing pen. If cells overlap, show them as they are and remember that no cell in a close tissue has re-entrant angles. The few exceptions to this rule are in open tissues, like the leaf mesophyll or loose pith tissues, and are easily recognized when you meet them.

Lastly, do not try to make drawings too extensive. Select a few important cells at a significant point of the tissues and concentrate on representing them exactly as you see them. Do not leave them looking like an isolated group, but indicate in your drawing, by leaving a few unfinished cells at the edges, that they are part of a continuous tissue.

Avoid the type of drawing which consists of a long chain of cells, ostensibly showing the structure right across a section. Such drawings are very difficult to interpret after a lapse of time and the object is much better attained by a series of drawings of selected groups of cells.

An excellent method of acquiring a correct idea of the shapes of cells is to commence by using a disc ruled in squares placed in the eye-piece. Rule a series of similar squares on the drawing paper and then draw several groups of cells, carefully plotting their shapes by means of the reference squares in the eye-piece. The accuracy of observation thus induced will have a good effect on all the subsequent drawings.

Section 3. Methods and Reagents

A. PREPARATION OF MATERIAL

Material should be preserved while in as fresh a state as possible. However obvious such a precaution may appear, experience shows that it is often neglected and plants are allowed to languish in the laboratory for days before being dealt with. " Kill your material before it is dead " is not so Irish as it sounds.

Where only the external form or the gross anatomy is to be examined this neglect does not perhaps matter very much, but it renders, for example, the fine structures of reproductive organs, or of Thallophyta, quite useless for examination.

In the vast majority of cases preserved material is intended to be cut into sections, or else, if very small, mounted whole to form microscope preparations. We will therefore deal first with the preparation of the material for such purposes.

There are three different processes involved in the preparation of material, which may be performed separately or, in some cases, in one combined operation. These three are **fixing, hardening** and **preserving.**

Fixing implies the most rapid killing of the cells in such manner as to produce the minimum of alteration in their structure. The term applies, therefore, chiefly to the protoplasmic structures.

Hardening is required by soft material to make the cutting of sections possible.

Preserving implies storage in some liquid which will keep moulds at bay (no easy matter) and which will not macerate the tissues or make them unduly brittle if they are left in it for a long time.

The formulae for the liquids recommended for these purposes are given in the Appendix I, page 454, which should be consulted. We will here confine ourselves to a few general remarks on their uses.

1. Fixing

For the general purpose of fixing material for class study nothing is better than a mixture of 70 per cent. Alcohol and 6 per cent. Formalin. It is better and cheaper than plain methylated spirits and, with many Cryptogams in particular, the fixation is quite good. It also hardens and preserves the majority of tissues satisfactorily.

The obvious query that will be made by most students on reading the last sentence will be, " Why then bother about any other method ? " and the answer is that in the majority of cases there is no need to do so. In the remainder, either there are special structural peculiarities or it is necessary to have a better fixation of the protoplasmic structures, or it is essential to avoid the shrinkage which takes place to some extent with all alcoholic fixatives.

Experience alone will reveal the cases where another method must be adopted, but as a general guide one may say that more careful fixation and preservation will be required by all the smaller and more delicate Algae and Fungi, as well as by all material in which the details of protoplasmic structures are required, such as sexual reproductive structures, and by material for the study of nuclear structure or division.

The action of fixatives should be as rapid as is compatible with safety to the cell contents. **Cytoplasm** is best fixed by Chromic acid (Chromic anhydride), Potassium dichromate, Osmic acid (Osmium tetroxide) and Picric acid. **Nuclei** are best fixed by Osmic acid, Acetic acid or Formic acid. The various mixtures recommended are supposed to give a balance of these qualities as far as possible.

Nevertheless, all these substances have their defects, and there is no universally perfect fixative.

Alcohol by itself is not a good fixative, but it aids the penetration of other reagents. Osmic acid is exceedingly rapid and effective in action, but it does not penetrate well and it is apt to make cell contents unstainable if allowed to act for too long. Chromic acid has the same drawback if not washed out sufficiently well after use. Acetic acid causes swelling and partial solution of the cytoplasm. Formalin is slow and does not harden.

The choice of an aqueous or alcoholic solution depends on the nature of the material and on convenience. Aqueous fixatives on the whole have a better action, but they are slower, are more troublesome to wash out and they do not penetrate so well as alcoholic fixatives.

There are one or two further points of importance. Firstly, material for fixation must be absolutely fresh. Secondly, the pieces must be cut *as small as possible*, never more than a few millimetres cube, if the best results are to be obtained. Thirdly, a relatively large bulk of fluid must be used. Lastly, after fixing and washing, the material must be brought up to strong Alcohol *by stages*. Starting from water, the following grades should be used in succession and kept made up in stock for the purpose: 10, 20, 30, 50, 70, 90 per cent. Allow about one hour in each strength.

2. Hardening

Fresh material or material fixed in an aqueous liquid is often too soft to admit of good section cutting. If it is to be mounted whole this does not matter. On the other hand, for most botanical material strong Alcohol, 70 per cent. or upwards, does all the hardening required. The 70 per cent. grade is perhaps the best all-round agent, and absolute Alcohol need not be employed unless complete **dehydration** is necessary as a preliminary to embedding in paraffin wax (see under " Embedding ") or before certain special staining processes (see under " Staining ").

Alcohol of too strong a grade will shrivel delicate material unless it has been very well fixed beforehand, or if such Alcohol is mistakenly used as a preservative fluid.

3. Preserving

Some fixing solutions, *e.g.*, Formalin-alcohol, " Universal " Fixative or N.K.L. may be allowed to act for long periods and so may be used also as preservatives, a practice which has all the beauty of simplicity. Where necessity dictates the use of some other fixative, the material, after washing if necessary, must be transferred to another liquid for preservation. For botanical material 70 per cent. Alcohol is indubitably the best all-round preserving medium, except for some of the more delicate lower organisms, where 5 per cent. Formalin, Formol-glycerine, or Alcohol-water-glycerine are better.

4. Maceration

Apart from the procedure of section cutting, dealt with below, there is another method of preparing material for microscopic examination, namely **maceration.** This was the method most popular with the early plant anatomists before the section-cutting technique was devised. It survives in modern practice only to a limited extent, to aid the recognition of the chief types of cells by beginners. It has been unjustly neglected, and there is no doubt that all observers should more frequently resort to the examination of macerated tissues to amplify and correct impressions derived from sections.

The principle of maceration is to dissolve the middle lamella of the cell wall, thereby allowing the cells, whole and undistorted, to fall apart. The classic fluid for this purpose is Schulze's solution, which is, however, open to the objections of being both fierce and dangerous. The Ammonium oxalate method is to be preferred. Harlow's method macerates and also delignifies tissues, if carried far enough, and this may be an advantage with some dense woods. Probably the best general method is to soak fresh material in 5 per cent. Chromic acid overnight, and then tease out on a slide.

5. Mounts of Entire Objects

It is often necessary, especially with lower organisms, to make preparations of the entire plant or of a substantial part of it. If the material is very delicate it may be very difficult to dehydrate it without serious shrinkage and an aqueous medium, such as Glycerine Jelly or else a mixture of Glycerine and Gum, is preferable for mounting. Lactophenol is also very suitable for either Algæ or Fungi, but it does not set solid and permanent preparations must be ringed.

If the material can be dehydrated by the slow evaporation of Glycerine, so that it can be transferred to absolute Alcohol, then a resinous medium may be used. A 5 per cent. solution of Canada Balsam in Dioxane, which is allowed to concentrate by evaporation, is sometimes recommended, but the refractive index of balsam is too high for unstained objects.

Alternatively a 5 per cent. solution of Venetian Turpentine in absolute Alcohol may be used and allowed to thicken by evaporation, or Wallis' Gum Sandarac medium (see p. 26). Both have low refractive indices and show details of structure very well.

B. SECTION CUTTING

For histological examination of plant tissues it is usually necessary to cut sections of the material, and for this purpose a razor of the old " cut-throat " pattern is generally employed. Certain types of holders have been put on the market by means of which safety razor blades can be used for the work, but in general these fine blades are not so satisfactory as the more robust ones. The razor used should be flat-ground, that is to say, the blade in section should have its two surfaces converging in a V, the sides of which should be flat. Such razors are stocked by all laboratory supply agencies. Hollow-ground razors, in which the sides are concave, are not suitable for botanical work.

Various methods will appeal to individuals as to the most convenient way of holding the razor, but one very suitable way is to turn back the handle at right angles to the back of the blade and grasp it so that the handle passes between the second and third fingers, with the thumb pressed against the milled surface of the thick shank of the blade, the second and third fingers against the back of the blade and the third and fourth fingers against the prolongation of the blade, beyond the point of its attachment to the sheath (Fig. 2). In this way the razor is firmly held, while at the same time allowing for free movement.

For section cutting both the material and the razor blade must be flooded with either water, for fresh material, or Alcohol, for preserved material. The piece of material should be long enough to be firmly held, and should be held against the side of the first finger of the left hand by means of the thumb. The first finger should be kept as straight as possible, while the thumb is kept well down below the surface of the material out of the way of the razor edge.

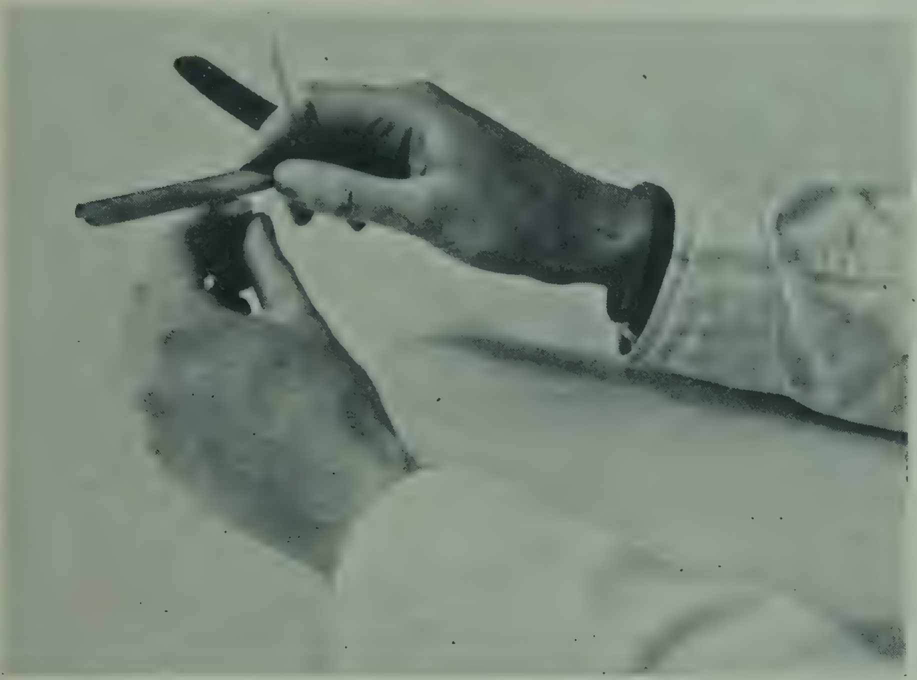

FIG. 2.—Method of cutting a hand section.

Now take the razor in the right hand, holding it in the way described, and place it on the first finger of the left hand, more or less at right angles. Draw it across the top of the material in such a way as to give the material a drawing cut. Special care should be taken when cutting to see that the material is not cut obliquely, which may easily happen if the material is not kept up level with the left first finger. Examine the material after cutting to see that the cut surface is truly at right angles to the long axis or parallel to it, as the case may be. However thin a section may be it will be quite unsatisfactory if it has been cut obliquely. By slightly and progressively increasing the pressure with the razor on the first finger, a number of sections can be cut without moving the material or the thumb. If these sections are reasonably thin they will adhere to the upper surface of the razor blade.

It is best to start cutting with the edge of the razor *on top of* rather than *against the side of* the material. It is much easier in this way to get a really thin section, while it is not usually necessary that a section should be complete, so long as it includes at least a portion of the tissues from the centre to the circumference.

Put some of the liquid used to moisten the material into a watch-glass and with a fine paint brush sweep the sections off the razor into the liquid. A good test for a thin section is that it will bend over when lifted with a brush. A section lifter can be used if preferred, though a brush is usually found most convenient. A selection of the thinnest sections should be sorted out and put into a second watch-glass of the same liquid.

The razor should be dried and stropped immediately after use to preserve the keen edge of the blade. Razors are improved by frequent stropping, first on the leather side of the strop, on which a little oil, soap or stropping-paste is rubbed in, and secondly, on the canvas side. In stropping, care must be taken that the turn is made over the back of the blade and not over the edge. The latter method not only cuts the strop but turns back the edge of the blade. If the razor becomes very blunt it may be sharpened by oblique strokes, edge foremost, on a very fine oil-stone, but unless this is carefully done the set of the blade may be damaged and the razor made worse than it was before. A good razor is one of the most valuable tools of the botanist, and it should be treated with the greatest care. A good test for a razor edge is cutting a hair. Hold a single hair by its end and draw it loosely along the edge. A good razor should cut through the hair easily.

Some material, such, for example, as the lamina of a leaf, may be too thin or too small to be cut easily in the way described, but by sandwiching it between two pieces of pith it can be cut without difficulty. **Elder pith** is the most suitable material for the purpose, since it is soft yet rigid and is easy to cut. Selected pieces about 2 in. long should be used. With a sharp scalpel cut a piece out by making a cut down the centre and then another cut through one side to meet it. In this way a segment is cut out. In the cut insert the material, which should not be wider than the diameter of the pith, and replace the segment cut out. The block so formed is then used in the usual way and sections are cut through both the pith and the material together. After the sections have been placed in a watch-glass of liquid, the pith sections can be removed easily and the sections of the required material left behind. If elder pith is not available, the pith of the Jerusalem artichoke, or carrot hardened in strong Alcohol, or the stipe (stem) of the brown seaweed *Laminaria digitata* hardened in 50 per cent. Alcohol, will be found quite satisfactory. As a last resort potato may be used, although owing to the starch grains in the cells this will be found less suitable for section cutting.

When pith is used it may be found convenient to hold it in a spring clothes-peg, which grips the pith firmly and may be laid down on the bench to leave both hands free without disarranging the material.

The **hand microtome** is a useful instrument to aid the cutting of sections by hand. It consists of a brass tube, held in the left hand or mounted on a support, in which the material is held by means of a screw clamp (Fig. 3). At the upper end is a flat table, usually covered with glass, on which the razor blade slides, and at the lower end is a screw by means of which the material and the clamp are together raised in the tube. The material may be used either with or without a pith support. It is clamped in the tube with about 1 mm. projecting above the level of the glass table and is cut off smooth with the razor. Now turn the screw about one-quarter turn, which will raise the material slightly, and cut again. A little practice will soon teach the student how much he should raise the material each time in order to get sections of a satisfactory thinness.

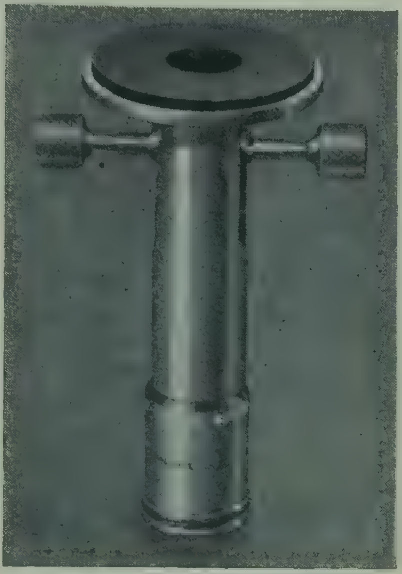
FIG. 3.—A good form of hand microtome.
(*Courtesy of the Bausch & Lomb Optical Co. Ltd.*)

This method has several advantages. It enables a number of sections to be cut rapidly, at approximately the same thickness, which may be very useful for supplying a class or when a series of sections through an organ is required. Further, it enables entire sections to be cut, and perhaps most important of all, it enables one to cut good sections of very small or delicate objects.

Any of the above-mentioned materials may be used to support small objects in the clamp, or material may be embedded in melted paraffin wax and a block of this, containing the object, cut out when cold and used in the microtome.

A word of warning is necessary in this connection. Avoid the type of hand microtome in which the razor slides in a clamp on rails. These are suitable for zoological material but are of no use for plant material.

C. STAINING METHODS

In order to preserve permanently sections of botanical material for microscopic examination it is necessary to mount them in a transparent medium which will not evaporate and whose refractive index is as near as possible to that of glass. For this purpose Canada Balsam, which is obtained as a resin from the conifer *Abies balsamea* is most generally used. This resin dissolves in various solvents, of which Xylol, Benzyl alcohol and Cellosolve are the most convenient. The effect of these solvents on plant tissues, however, varies. Xylol will not mix with water, and forms a cloudy emulsion even in the presence of minute traces. Benzyl alcohol, although slightly more tolerant to water, behaves in much the same way. Cellosolve will mix freely with water, but dilute Cellosolve causes cloudiness in the Balsam.

It follows, therefore, that a process of dehydration is necessary before the material can be mounted. This is generally effected by transferring the material through increasing concentrations of Alcohol until all the water has been removed and the material remains in pure Alcohol. Xylol and the other solvents mentioned will mix with pure Alcohol. The transfer from Alcohol to Xylol causes the material to become transparent, and it is said to be **cleared.**

Certain other fluids can be used instead of Xylol for this purpose. The most popular are: Clove Oil, thin Cedarwood Oil, Oil of Cajeput and Terpineol. Clove Oil has the property, advantageous in some cases, of dissolving many of the aniline dyes. It can thus be used for differentiating the staining of sections as well as clearing them.

A cleared specimen can be transferred directly to a Balsam solution and subsequently, by the evaporation of the solvent, it becomes embedded in a hard resin. One of the results of clearing is that by rendering the specimen transparent it makes it impossible to pick out the finer detail under the microscope, so that a process of **staining** is generally employed before the specimen is mounted.

The staining of material serves two purposes. Firstly, it makes the specimen easier to see, and secondly, by the careful selection of the dyes used it is possible to differentiate between different tissues or structures, according to their reaction to various dyes. For example, certain dyes show a greater affinity for wood than for other types of tissue, while with other dyes the reverse is the case. From this point of view we may recognize two main groups of dyes: the basic dyes which show a greater affinity for woody tissues, and the acidic dyes which do not. For sections containing woody tissue, therefore, a method of **double staining** is generally adopted, which by the combination of an acidic and a basic dye of contrasting colours, distinguishes the woody tissue clearly from the non-lignified cells.

The parts of a single cell may also be differentially stained, for certain dyes show a great affinity for the chromatin of the nucleus while others do not. By the careful use, therefore, of certain dyes it is possible to stain the chromosomes, the nuclear spindle and the cytoplasm of a cell different colours, with the result that these structures become clearly visible and distinct.

Many different stain combinations have been suggested for botanical work, but, for one reason or another, some have become especially popular, and we shall have to consider in detail the methods employed in each case.

The dyes used in the staining of botanical material fall into three main classes. Firstly, there are the Haematoxylin solutions, which are compounded either with pure crystalline Haematoxylin or with Haematein and sundry other chemicals, to make a dye which is blue in alkaline solution and red in acid. The basis of these Haematoxylins is Logwood (*Haematoxylon campechianum*). Secondly, there are the Carmines, which either singly or in combination with other substances stain plant material red. Thirdly, there is the large range of aniline dyes.

In the case of the Haematoxylins the dye is precipitated in the cell walls, either directly or through the agency of a mordant, and the stain so formed is not soluble either in water or Alcohol. The same is true to some extent of the Carmines. On the other hand, the aniline dyes are readily soluble in water or Alcohol. Some are even soluble in Clove Oil or Cellosolve. It follows, therefore, that these dyes may wash out during the processes of dehydration and clearing, and it is usually necessary when using aniline dyes to overstain material to allow for the stain lost during these processes. This can be avoided by using a non-solvent for stains, *e.g.*, Tertiary Butyl Alcohol, to dehydrate the sections ; or by dissolving the stains in the liquid used for dehydration.

Mordants, that is to say solutions of certain substances which help to retain the dye in the tissues, are sometimes used in botanical work, as they are commonly used in the dyeing of fabrics. Picric acid, Chromic acid, Potassium permanganate, Iodine and Aluminium sulphate may be mentioned, and a 1 per cent. solution of one of these substances may be used with advantage before staining. It will be noticed that the first two of these are components of common fixatives, and it follows that where such a fixative has been used, sections cut from the material stain more readily with aniline dyes.

1. Histological Stains

Ehrlich's Haematoxylin and Safranin.—Very thin sections of the material are placed in a watch-glass containing a little 50 per cent. Alcohol and the thinnest sections transferred with a fine paint brush to a microscope slide bearing a small drop of distilled water. A few drops of Ehrlich's Haematoxylin are added and the sections left for about five minutes. The stain is then washed off with water and the section examined in water under the low power of the microscope. If both the lignified and non-lignified tissues appear to be stained it will be necessary to remove the excess of stain from the lignified tissue. This is done by adding a drop of a 1 per cent. solution of Hydrochloric acid in 25 per cent. Alcohol. This process will be completed very rapidly and should be watched under the microscope. Immediately it is done the acid must be washed off with water and the slide inverted over a bottle of 50 per cent. Ammonium hydroxide. In this way the water will become alkaline and the dye will turn blue. In certain parts of the country where the tap water is alkaline, *i.e.*, " hard water," it will probably be found sufficient to wash the section in several changes of tap water, and the Ammonium hydroxide may be omitted.

After " blueing," the sections should be inspected again to see that the blue colour persists only in the non-lignified tissue. If this is not the case the treatment with acid must be repeated. When it is considered that the Haematoxylin staining is satisfactory, the section should be transferred first to 50 per cent. Alcohol and then into 1 per cent. Safranin dissolved in 50 per cent. Alcohol. The stain should be allowed to act for ten to fifteen minutes. The excess of stain should then be washed off with 95 per cent. Alcohol.

Now get ready two small watch-glasses. Incline the slide and quickly wash the sections into one watch-glass with a stream of absolute Alcohol. Half fill the second watch-glass with absolute Alcohol and, with the aid of a needle, preferably of triangular section, pick up the sections one by one and place them in the absolute Alcohol in the second watch-glass. Place this watch-glass on a dark surface and drip in Xylol drop by drop, taking care that during the process none of the sections come up to the surface. Should the mixture become milky it indicates either that the sections have not been properly dehydrated or that the absolute Alcohol contains traces of water. It should be noted, however, that there is a grade of Xylol on the market which will not mix with 100 per cent. Alcohol, and care should be taken that a grade suitable for mixing with absolute Alcohol is specified when ordering.

After the sections have remained in this mixture of Alcohol and Xylol for about a minute it will be seen that they have begun to clear. Empty and dry carefully the first watch-glass and half fill it with pure Xylol. Transfer the sections quickly, one at a time, to the pure Xylol, again taking care that the sections do not float to the surface. In a very short time the sections will be cleared. Should too much Safranin be found to be present, and the non-lignified tissues are seen to be stained red when the sections are examined in Xylol, they may be replaced in absolute Alcohol or a mixture of Alcohol and Xylol, which will remove the excess of stain. If this has to be done the sections must again be cleared as before.

When the staining is considered correct, clean a glass slide and place on the centre a small drop of Canada Balsam. Quickly transfer to it with a needle two or three of the best stained and thinnest sections and push them well down into the drop. A clean cover slip is best obtained by keeping the stock in a jar of absolute Alcohol and withdrawing one and holding it in a flame for a second to dry it. Take the cover slip in the forceps or else hold one side of the slip up with a needle while gently lowering it obliquely on to the drop of Balsam. In this way it is easy to prevent air bubbles from being locked under the glass cover.

The slide should then be dried by placing it on a hot stage, which can be conveniently made by enclosing a low (20 to 40) wattage electric lamp in a small wooden box, with a sheet of glass for a lid. As soon as the slide is dry it should be carefully labelled, giving the *name* of the specimen, the nature of the *tissues* cut, the *stains* used and the *date* when the section was made.

Safranin and Spirit (*Aniline*) *Blue.*[1]—In this double stain two aniline dyes are used, Safranin, which stains the lignified tissues, and Spirit Blue, which stains the non-lignified tissues. The process is more difficult to carry out than the last because both stains are soluble in Alcohol, and consequently, while staining with the second dye, the first is to some extent dissolved out in the Alcohol used to make up the second stain. It is customary to use Safranin made up in 50 per cent. Alcoholic and Spirit Blue dissolved in 95 per cent. Alcohol, though some workers prefer Safranin made up in water. Improved differentiation in difficult cases is obtained if some Picric acid is added to the Spirit Blue. It is a good general rule to stain first with the stain which is made up in the lower grade Alcohol and put sections into the second stain when the appropriate higher grade of Alcohol is reached during the process of dehydration. In this way a transfer up and down through grades of Alcohol is avoided, and less of the first stain is lost in the process. **A scheme for staining plant material** is given on page 30 and should be consulted while reading the following sections.

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[1] Spirit Blue is the correct name now given to those acid blues of the aniline series which are soluble in Alcohol. Soluble Blue is the name applied to Cotton Blue, Methyl Blue and other water-soluble blues. Since many samples of aniline dyes are compounded with a "bodying" material, often china clay, the actual strength of a 1 per cent. solution will depend upon the quantity of "body" which is present in the sample.

Assuming that the material has been preserved in about 70 per cent. Alcohol, the selected sections are placed immediately into a drop of Safranin stain and left there for at least ten minutes. Longer staining, if possible, is better, since the excess can always be washed out in Alcohol, and by this method a more intense staining is obtained in the lignified tissues.

The excess of stain is washed off with 95 per cent. Alcohol, and the sections examined in a watch-glass under the low power of the microscope, taking care that the Alcohol does not dry up during the process. When most, but not all, of the stain has come out of the non-lignified tissues a drop of Spirit Blue is added and allowed to act for about five minutes. This is then washed off with fresh 95 per cent. Alcohol and the sections transferred to a watch-glass of absolute Alcohol. The clearing of the preparation and the subsequent treatment are the same as above. If a section is over-stained with Spirit Blue, it can be put back into absolute Alcohol, but it must be realized that treatment with absolute Alcohol will bring out both stains, so that it is important to arrange by trial the times of staining to obviate, as far as possible, the necessity for washing out any Spirit Blue after the section has been once brought into Xylol. The time needed will depend on the fixative and on the sample of dye used and also on the speed at which the worker manipulates the Alcohol and Xylol changes.

If Picric acid has been added to the Spirit Blue, sections may be transferred to this stain directly from the Safranin without intermediate washing.

Gentian Violet (*or Crystal Violet*) *and Bismarck Brown.*—Crystal Violet is a pure substance and the chief constituent of Gentian Violet. Both violets are extremely soluble in water and in Alcohol. They are used for staining lignified tissues in histological preparations, and may be made to stay in the tissues better if the sections are first treated for a few minutes with a dilute solution of either Iodine or Picric acid or Potassium permanganate. Generally an aqueous solution of the dye is employed and allowed to act on the sections for at least a quarter of an hour. Time may be saved by transferring the sections directly to the Bismarck Brown in 70 per cent. Alcohol at the end of that time and then, after about a minute, washing both out together with 95 per cent. Alcohol. After this the sections are quickly washed in absolute Alcohol in a watch-glass, and then transferred at once to a second watch-glass of absolute Alcohol. Xylol is added immediately, and in this mixture any excess of stain can be removed since in this the stain comes out relatively slowly. It should be noted that Gentian Violet is soluble in Xylol and that therefore in an Alcohol-Xylol mixture the Gentian Violet comes out more rapidly than the Bismarck Brown. In a well-prepared slide the lignified tissues should be stained violet and the rest of the cell walls brown. Nuclei and starch grains also take up the violet colour.

Gentian Violet and Erythrosine or Light Green.—Very pleasing histological preparations may be made by first staining the sections in Gentian Violet, which for this method may be made up in 95 per cent. Alcohol, and then dehydrating and clearing the sections as before described. Excess of Gentian Violet may be slowly removed in Clove Oil, but since this process is relatively slow, much overstaining in Gentian Violet is unnecessary. The sections are then placed on a slide in a drop of either Erythrosine or Light Green dissolved in Clove Oil. This is allowed to act for two or three minutes. The excess of stain may be drained back into the bottle and the sections washed in a few drops of clean Clove Oil. As soon as the excess of stain has been removed, they are placed in Xylol and mounted in Balsam. By using the second stain dissolved in an anhydrous solvent such as Clove Oil, and applying it last in the sequence, all risk of losing the stain during dehydration is avoided.

It is possible to mount straight from Clove Oil into Balsam, but such mounts will dry slowly since Clove Oil is not so volatile as Xylol. Well-stained sections will have the lignified tissues stained violet, and the non-lignified stained red or green according to which second dye is used.

Basic Magenta (Fuchsine) and Methyl Green, a Single Solution Double Stain.—The ultimate simplification of the process of double-staining is the use of both stains together in one solution. This, however, is only possible in favourable cases and with certain combinations of stain, and the above is recommended as one which has been extensively used.

Mix equal quantities of 1 per cent. solutions of Basic Magenta and of Methyl Green (Iodine Green) in 50 per cent. Alcohol. The sections are simply placed in the stain for several minutes, then washed quickly in 95 per cent. Alcohol and transferred immediately to Clove Oil, which slowly completes the process of differentiation begun by the Alcohol. Wash in Xylol and mount in Balsam.

The resulting coloration and the success of the differential staining varies with the material used, but in sections of vascular cryptogams the lignified tissues are usually stained reddish-purple and the cellulose tissues bluish-green.

Simplicity and rapidity are the chief recommendations of the process, but like most short-cuts it is liable to be disappointing.

2. Cytological Stains

For **cytological work** the problem is more complicated, since the delicate structures in the cell will not stand up to the drastic changes from water to 50 per cent. Alcohol and then to 95 per cent. Alcohol recommended for histological work. Moreover, the purpose of the stains here is mainly to bring out the minute details of the nuclear structure. Only material which has been properly fixed in the living state can be used for this purpose, and, if nuclear configurations are required, it may be necessary for the material to be fixed at a particular time of the day or night when divisions of the nuclei are most frequent.

The following staining methods are among the more simple and commonly used for this work :—

Heidenhain's Haematoxylin and Erythrosine.—In this method, use is made of a mordant for the Haematoxylin, and the sections are first placed for several hours in a 2 per cent. solution of Iron Alum. They are then quickly washed and placed in Heidenhain's Haematoxylin for about an hour. The section is then washed with water and examined under the low power of the microscope. If correctly stained, chromatic material should be black, while the cell walls are grey. Should there be too much stain in the tissues, the sections may be de-stained by the re-application of the mordant, which dissolves excess of the stain. The removal of stain may be watched under the microscope, and when it has proceeded far enough, the Iron Alum is thoroughly washed out with water. The sections should then be taken up through a succession of Alcohol grades. The number of changes necessary will depend upon the delicacy of the material. Usually 25, 50, 75, 95 per cent., and absolute Alcohol will be found satisfactory, though for delicate material intermediate grades may be necessary. The sections are then cleared in Xylol, as already described.

If, in addition, a plasma stain is required, though many cytologists find this unnecessary, the sections can be treated for a few minutes with Erythrosine in Clove Oil before mounting in Balsam. Sections stained in this way should have chromosomes, spindle fibres and nucleoli stained black, cell-walls grey, and the cytoplasm red.

Gentian Violet-Iodine.—Cytological material to be stained by this method should be fixed in Fleming's solution for two to six hours and then thoroughly washed for at least an hour in water. It is then graded-up through 10, 20, 30 and 50 per cent. Alcohol, and bleached in a solution of 10 c.c. of " 20 volumes " hydrogen peroxide, in 100 c.c. of 70 per cent. Alcohol, for a few minutes. The purpose of this is to remove the black colour produced by the osmic acid in the fixative, and for all cytological work material fixed in a solution containing Osmic acid must be so treated before staining, whatever method is used. The sections are then brought back to water through successive grades of Alcohol and stained for about five minutes in Gentian Violet made up in water. The sections are brought up again through similar grades of Alcohol to 70 per cent. and then put in a solution of 1 per cent. Iodine and 2 per cent. Potassium iodide in 70 per cent. Alcohol and allowed to remain for a few minutes. The Iodine solution is followed by 85 per cent. Alcohol and afterwards by 95 per cent. Alcohol. The sections are then transferred directly to Clove Oil, which mixes with 95 per cent. Alcohol.

In the Clove Oil the excess of stain will be removed. From Clove Oil the sections may be transferred to Xylol before mounting in Balsam. Well-stained sections should have the chromatin and spindle fibres stained violet, but the cell walls and cytoplasm unstained. Many cytologists consider this method is an improvement on the older Heidenhain's Haematoxylin technique, because the violet chromosomes are clearly recognizable and more sharply stained, compared with the black, often " woolly " staining with Haematoxylin. Moreover, there is less risk of mistaking tiny particles of dust for chromatic material, which is quite possible when the latter is stained black with Haematoxylin.

Methyl Green-Acetic Acid.—This is a pure nuclear stain, and it is made by dissolving 0·5 to 1·0 per cent. of Methyl Green in 2 per cent. Acetic acid. It can be applied only to sections of fresh material which has not been fixed. These are laid in the stain for a few minutes, then rapidly washed in water and transferred to Glycerine on a slide. No washing out is needed, as only chromatin is stained. The stain is not permanent.

Aceto-Carmine.—The Aceto-Carmine technique is specially adapted for smear preparations where it is required to stain chromosomes in a nucleus without necessarily bringing out the details of the associated cell structures. Two methods may be mentioned. In the first, young roots of *Vicia faba*, the Broad Bean, which have been germinating for about five days, are used. Pieces about 5 mm. long are boiled in water and placed on a slide in a drop of Carmine solution. Then two needles are taken and while holding the end of the root with one, the other needle is used to split the root into several longitudinal strips. These strips are then placed separately on a fresh slide in a drop of Carmine and covered with a cover-glass over which a piece of filter paper is laid and the whole pressed hard down without any sideways movement. In this way the root tissue should be reduced to one-cell layers. In the cells so pressed out the chromosomes should be brightly stained red.

In the second method, longitudinal hand sections are made and transferred with a needle to a drop of Carmine solution on a slide. The thinnest sections are brushed carefully on each side to remove the injured cells, then covered and heated carefully for about a minute over a small flame, replacing any lost Carmine solution during the process. The sections are then cooled and the excess Carmine solution mopped up with blotting paper. No pressure is needed in the case of thin sections.

If permanent preparations are required, the sections should be rapidly washed in 45 per cent. Acetic acid and then transferred to 95 per cent. Alcohol. From this they may be dehydrated with absolute Alcohol, cleared in Xylol and mounted in Balsam. The cytoplasm is largely destroyed by this method, which is an advantage for chromosome observations.

Feulgen Method for Root Smears.—This is an application of Schiff's reaction for aldehydes and is equivalent to a microchemical test for nucleic acid. It gives a pure chromatin stain and is excellent for mitotic figures. The stain used is a 1 per cent. solution of Basic Magenta which has been decolorized by adding carefully a solution of Potassium metabisulphite until the red colour has just disappeared. A residual yellow colouration often remains but this may be ignored.

For material, use root tips of Onion (*Allium cepa*), preferably gathered about mid-day, for the maximum number of mitotic figures.

(i) Ten per cent. of conc. Hydrochloric acid in a tube, give two changes, allowing 3 to 5 minutes in each, then leave in a third change for 3 minutes.

(ii) Heat in this change of Hydrochloric acid to *exactly* 60° C. and hold at this temperature for *exactly* 4 minutes. This is done by placing the tube in a fairly large beaker of water at the correct temperature and maintaining it by judicious short periods of heating with a Bunsen burner.

(iii) Cool by draining off the warm acid and then adding a little cold 10 per cent. Hydrochloric acid. Drain off the acid.

(iv) Add just enough reduced Magenta solution to cover the roots, cork and leave from 30 to 60 minutes, giving an occasional gentle shake to aid diffusion.

(v) Drain off the Magenta and wash the roots for 10 minutes in running water to darken the stain and remove most of the excess dye. Drain.

(vi) Cover with dilute SO_2 water. This is prepared by taking 2·5 c.c. of a 10 per cent. solution of Potassium metabisulphite and 5 c.c. of 10 per cent. Hydrochloric acid made up to 100 c.c. Leave for 5 to 10 minutes. This removes any diffuse staining of the cytoplasm. Roots may be left at this stage more or less indefinitely.

(vii) Place a root on a slide and cut off the dark purple extremity (0·5 to 1·0 mm.) by means of a scalpel. Remove the adult portion and gently split the remaining tip into two or three pieces by careful teasing with a needle, taking care to avoid letting it dry up. Add a drop of dilute Glycerine and tap the cover-slip smartly with a wooden instrument to separate out the tissues.

3. Stains for Thallophytes

Because of their delicate nature, Algae and Fungi need rather different treatment from higher plants. Many of them are badly distorted when brought up through grades of Alcohol and cleared in Xylol, unless this is done extremely gradually, and, if an aniline dye has been used for staining, it frequently happens that no stain remains when the material has finally been successfully mounted in Balsam.

To obviate this difficulty other mounting media are generally used, of which Lactophenol, Glycerine, or Glycerine Jelly are the most popular. Although sometimes ordinary histological stains can be satisfactorily used, certain special stains are frequently substituted.

Lactophenol and Soluble (*Cotton*) *Blue*.—This method is suitable for both Algae and Fungi. Material preserved in Alcohol should be brought down slowly into water, material in Formalin should be put straight into water.

Take a clean slide and place on it a drop of Soluble Blue dissolved in Lactophenol. Carefully immerse the material in the stain with the aid of a needle and warm the slide gently over a small flame or electric lamp so that it steams but does not boil. Keep warm for 30 seconds or more according to the nature of the material. Wash off the excess of stain with warm Lactophenol.

Take a second slide which has been carefully cleaned to remove grease and put a small drop of Lactophenol in the centre. Transfer the stained material, taking care that the minimum amount of liquid is taken along with it. Take a clean dry cover-glass and gently lower it on the drop of Lactophenol, taking care that no air bubbles are trapped during the process. It is important that the Lactophenol should not exude beyond the cover-glass when this is pressed gently down.

If it is intended to keep the slide permanently it must be sealed by the application of some fluid which will form an impervious film round the edge of the cover-glass. This is done by ringing (see p. 26), and for this purpose a round cover-glass should have been used. Should the slide be intended for use with an oil-immersion objective, it is important that the medium used for final ringing be insoluble in Xylol, *e.g.*, Gold Size, otherwise the Xylol used to clean off the oil will gradually damage the sealing and eventually spoil the slide.

Picro-nigrosin.—This stain may be used very satisfactorily for Algae and Lichens. It is a combined stain and fixative. Since the stain is made up in water, the fresh material may be transferred directly to the stain for about five minutes. After staining, the material should be washed in water and then mounted either in Glycerine or in Glycerine Jelly (see p. 25). Picro-nigrosin will be found also to stain nuclei dark bluish-black in colour. In Lichens, the gonidia are stained blue-black, while the fungal hyphae appear yellow, having been stained with the Picric acid.

4. Stains for Special Purposes

Many special methods have been devised for staining difficult material or for special purposes, but a consideration of such methods is outside the scope of this book. Readers interested in the subject are referred to more advanced books on the subject, *e.g.*, " Plant Science Formulae," by the present authors.

Two methods of general interest will be briefly described here.

Polychrome-Methylene Blue.—For histological work, where a permanent preparation is not required, this stain will be found very useful. It is an aqueous stain, and sections can be transferred to it either from water or from 50 per cent. Alcohol. The stain should be allowed to act for about two minutes, the excess washed out with water and the section mounted in water or Glycerine. Lignified tissues are stained bright blue, pith is reddish-purple, phloem and other tissues purple or red.

Intra-vitam Staining.—It is sometimes desirable to stain material without killing the cells, and for this purpose certain non-toxic stains may be used. The concentrations of the dyes should range between 1 in 10,000 and 1 in 100,000. Neutral Red, Janus Green and Methylene Blue are specially recommended for the purpose. Only certain cell structures can be stained in this way. Nuclei are very seldom stained *intra-vitam.*

D. MOUNTING PREPARATIONS

1. Mounting Media

The use of **Canada Balsam** for mounting has already been described (see p. 18), but this resin possesses important defects, one of which is that the solution is frequently acid, and even if neutralized before use it may become acid again if exposed to light. The fading of many stains in permanent preparations is due to this. **Euparal,** which is a proprietary product, is neutral and remains so. It also has the advantage of mixing with 95 per cent. Alcohol, thereby obviating the use of Xylol.[1] It is, however, relatively expensive and is therefore unsuitable for use in large classes. Recently, experiments made with **Cellosolve** (Diethylene-glycol mono-ethyl-ether) have shown that this substance not only mixes freely with water and Alcohol, but also with Balsam. Sections, therefore, may be placed in dilute Cellosolve and the mixture gradually concentrated in a desiccator, after which they may be transferred to pure Cellosolve before being mounted in Cellosolve Balsam.[2] In this way both Alcohol and Xylol are avoided. The chief disadvantage of Cellosolve is that it readily dissolves stains and therefore, if slow concentration of the solvent is adopted, there is a considerable risk that, by the time sections are mounted, they may be devoid of stain. The remedy for this is to stain *after* dehydration, with stains dissolved in Cellosolve, in the same way as with stains in Clove Oil (see under Gentian Violet and Erythrosine, p. 20).

Glycerine Jelly is a mixture of Glycerine and Gelatine in the proportions of one part of Gelatine dissolved in six parts of water, to which is added seven parts of Glycerine and, after mixing, 1 per cent. of Phenol to prevent the growth of bacteria.

Material to be preserved should be stained in an aqueous solution of Eosin or other suitable water stain, and, after washing off excess of stain, should be placed in a dilute solution (about 10 per cent.) of Glycerine. The concentration should then be raised by the addition of more pure Glycerine. In the case of delicate material, such as Chlorophyceae, the 10 per cent. Glycerine should be allowed to concentrate slowly under a cover. When the material is in pure Glycerine, some Glycerine Jelly is melted over a water-bath and a drop placed in the centre of a warm slide. The watch-glass containing the material in pure Glycerine should be slightly warmed and the material transferred to the jelly with a slightly warm needle. A clean cover-glass is then gently lowered on to the preparation and gently pressed down with a needle. This should be done as quickly as possible, since the drop of Glycerine Jelly solidifies rapidly in the cold. It is also important that the size of the drop of jelly should be such that when the cover-glass is put on and pressed down, the jelly just flows to the edge and not beyond it.

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[1] Certain transparent plastics have been recently employed as mounting media. The best known of these is **clarite,** which has been extensively used in the United States. It is not at present obtainable in this country.

[2] This is not obtainable commercially, but is easily made by dissolving solid Balsam in Cellosolve to a syrupy consistency.

Sandarac Medium (Wallis).—A very useful medium for entire objects. It sets hard and its low refractive index permits the observation of unstained objects.

Dissolve Gum Sandarac 32 gm. in a mixture of Amyl Alcohol 64 c.c. and Castor Oil 4 c.c.

Tissues to be mounted in this medium should be quickly dehydrated in absolute Alcohol and transferred to Amyl Alcohol before mounting.

2. Ringing Slides

"Wet" preparations such as those mounted in Lactophenol, Glycerine or Glycerine Jelly, may be made more permanent by ringing them with a solution of Gold Size, Murrayite or Gum Dammar, followed by enamel. For this purpose a **turn-table** is employed. This consists of a circular brass plate, pivoted in the centre, which can be easily rotated. The brass plate is marked with concentric circles and is provided with a pair of microscope slide clips. Circular cover slips must be used. The finished slide is arranged on the brass plate so that its cover-glass fits accurately over the circle of the same size. A small quantity of Gum Dammar or Gold Size is taken with a fine brush and applied to the joint between the cover-glass and the slide, while the brass plate is rotated rapidly with the finger. In this way a ring is made sealing the whole joint. It is essential that the glass slide and cover be perfectly dry and free from exuded liquid, otherwise the sealing mixture will not adhere.

Several successive layers of Gold Size, Murrayite or Cellulose paints may be applied, time being given between two applications to allow the previous one to dry, but no heat must be applied, since this would cause the medium under the cover-glass to expand, thus spoiling the sealing. Subsequently enamel may be applied and, by the use of different coloured enamels, very attractive preparations may be produced. This is a refinement usually left to professional slide-makers.

3. Labelling

All slides should be carefully labelled. Suitable labels, about one inch square, may be bought which allow for a description of the object to be written on them. Sometimes it may be desirable to use two labels, one giving the name and systematic position of the object, with the stain and mounting medium used and the date, while the other is reserved for notes on any particulars of special interest in the specimen. The date when the slide was made and the stains and technique employed should always be noted, since this serves as useful information regarding the keeping qualities of different stains and media. Microscope slides should never be left about in the dust, or in a bright light, but should be preserved in boxes provided with slots or trays to take a definite number of slides. Where a large collection is accumulated it may become necessary to employ a cabinet of drawers to keep slides, in which case a card index should be made, giving the full particulars on the label, as well as the drawer in which the slide is kept. Slides should be returned to their place immediately after use, since prolonged exposure to light causes many aniline dyes to fade, thus spoiling the value of the slide.

E. EMBEDDING AND MICROTOME TECHNIQUE

1. Embedding

Some plant organs, particularly reproductive structures, are too small or too delicate to be readily prepared by means of hand sections, and the student must have recourse to the more elaborate method of embedding them in paraffin wax and cutting the sections on a mechanical microtome.

For this purpose properly fixed material is desirable, though it is not essential for many vegetative structures. In either case the first stage is **dehydration.** When an alcoholic fixative solution has been used it should be washed out by placing the material in 70 per cent. Alcohol. If the fixative used was aqueous, the material should be washed in water and then transferred successively to 10, 20, 30, 50 and 70 per cent. Alcohols, at intervals of a few hours. From 70 per cent. Alcohol it is advantageous to pass to 95 per cent. Alcohol before entering absolute Alcohol, where it must remain for at least twenty-four hours, preferably with at least one change of Alcohol. In order that the Alcohol should penetrate the tissues thoroughly at each stage it is important that any hard, unnecessary, outer tissue should first be removed and that the pieces of material employed should be not more than a few millimetres long in any direction.

Dehydration may also be performed with Acetone or n-Butyl Alcohol. The latter has the great advantage of being also a solvent of paraffin wax, so that no transfer to Xylol is required. It is not, however, completely miscible with water, so that the lower grades of the dehydration series, up to about 50 per cent., should be made up with either Acetone or Ethyl Alcohol, followed by a series of increasing concentrations of Butyl Alcohol, up to wax. Material is less brittle after Butyl Alcohol than after Xylol, which is often a desirable feature.

The second stage is **clearing,** which implies the replacement of the Alcohol by a solvent of paraffin wax. For this purpose the following are commonly used : Xylol, Chloroform, Cedarwood Oil and Dioxane. Mixtures with Alcohol in the ratios of 1 : 3, 2 : 2, 3 : 1 are used in succession, the material remaining a few hours in each, and finally left in the pure solvent for twenty-four hours. It is then transferred to a fresh lot of the solvent, placed in an oven heated to a constant temperature of about 55° C. and to this solvent shavings of Paraffin wax, melting at 52° C., are added until the mixture contains about equal parts of solvent and wax. Alternatively the material may be transferred to a series of tubes containing increasing amounts of wax in solvent. From the mixture the material is transferred to a small vessel containing pure melted Paraffin wax, and in this it must remain for at least twenty-four hours. This process is known as **infiltration.** Large, or specially hard structures take longer, and should be passed through one or two changes of pure wax. Small potted meat jars make good vessels for melted wax, and their lids are useful to keep out any dust.

A small quantity, about 1 per cent., of Ceresine mixed with the Paraffin wax will often improve the cutting of difficult material. Another valuable modification is the addition of Diethylene glycol distearate to the Paraffin wax. It is best to use a wax of fairly low melting point, say 49° C., and add between 20 per cent. and 40 per cent. of the distearate. This addition increases the toughness of ribbons cut from it and reduces the liability of material to splinter in cutting. The higher the percentage of distearate the harder is the resulting mixture.

Good microtomy depends on good embedding, and the smaller the piece of material and the freer it is from hard tissue the greater the probability of success. Too long a period in the oven, or any overheating, will render the material brittle and sections cut from it will break up.

Next comes the **embedding** of the material. Make a little mould of folded paper, in rectangular form, and fill it with clean, melted Paraffin wax. Drop the objects into the wax and arrange them suitably with warmed needles. Allow the wax to set hard on the top and then float the block in its mould on cold water to harden more quickly. Metal angles are obtainable to replace the paper mould. These can be arranged to form a box, and are placed on a piece of glass. Care must be taken to cover them with a film of Glycerine before adding the wax, to prevent it sticking to the metal.

2. Microtomy

The exact procedure in cutting the sections will depend on the type of microtome available and cannot be specified in detail. One or two steps are, however, generally applicable.

A portion of the wax block containing the material should be cut out with a scalpel and fixed to the block holder of the microtome, on which a drop of melted wax has been placed. This is best done by melting the surface of both the block and the wax on the holder with a warmed scalpel and quickly bringing the two wax surfaces together, after which the edges can be completely sealed by touching them in turn with a hot needle. The block must then be squared up so that it presents accurately parallel edges when viewed looking towards the holder, *i.e.*, at the end which will meet the microtome knife. This parallelism is essential if a ribbon of adherent sections is desired, as is usually the case when a series of sections through an organ is the object in view.

When a ribbon of sections has been cut it should be laid out flat on a piece of clean glass backed with black paper. Examine the ribbon with a magnifier and decide the portion of it which is most promising. Detach this in short lengths of not more than one inch by cutting the ribbon carefully across.

Prepare a carefully cleaned slide by rubbing over it a minute drop of Albumen Fixative (see p. 458). Draw a streak of distilled water with a brush along the slide, near the middle, pick up a portion of the ribbon with needles and lay it carefully on the water, avoiding crumpling, air bubbles and any irregularities as far as possible. Be careful to see that the shiny side of the ribbon, *i.e.*, that which was lowermost as the ribbon came off the microtome knife, is the one in contact with the water. If the wax block has been pared down small enough, as directed, it will be possible to mount several portions of the ribbon in parallel lines on the slide.

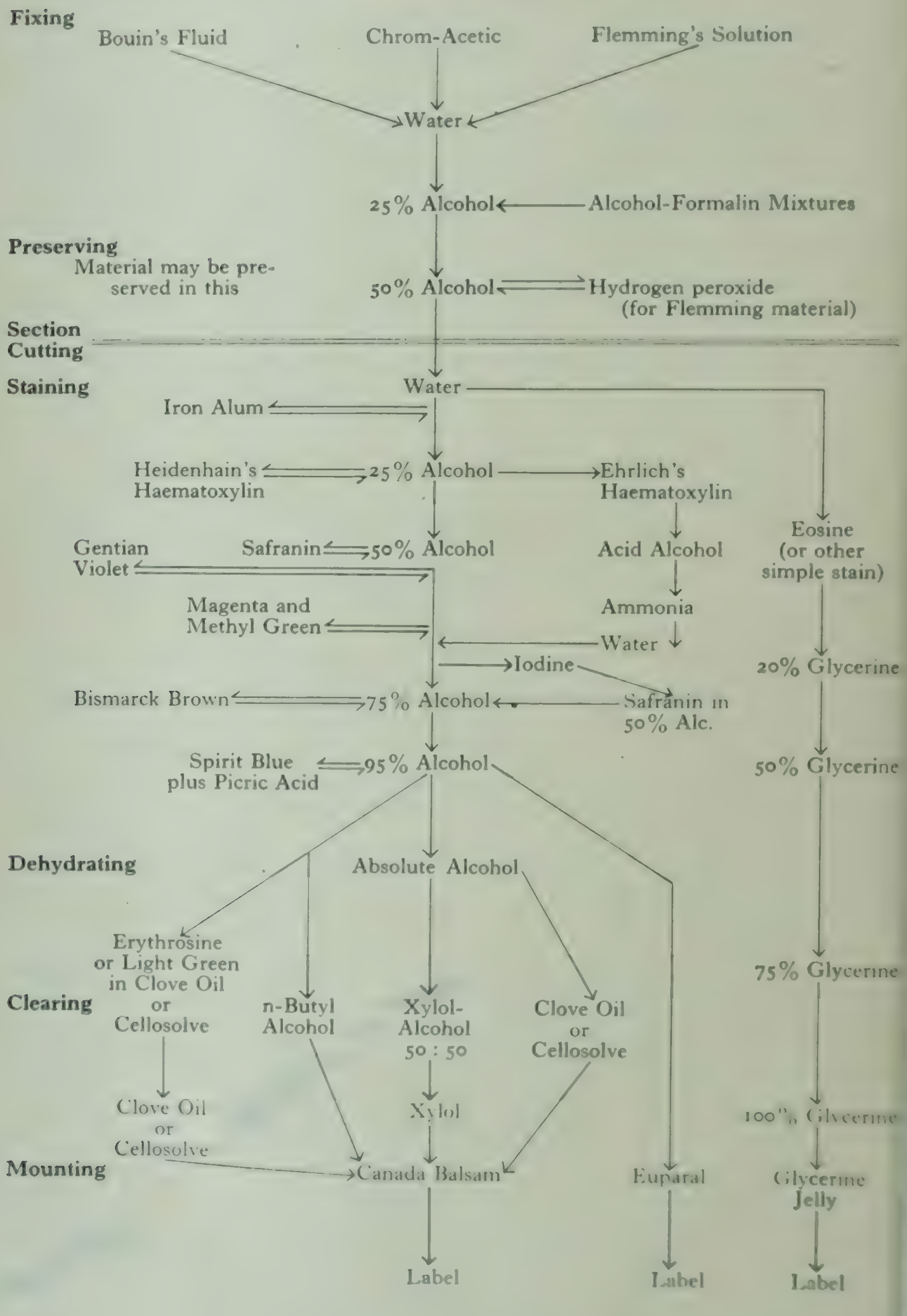
Add a few more drops of water to ensure that the ribbons are floating freely, and then warm the slide very carefully over an electric lamp. The sections will expand and flatten as they get warm, but heating must be stopped immediately this occurs. On no account must the wax be allowed to melt. If it does, the sections will not adhere to the slide because the wax flows underneath them.

Drain off excess of water and, if necessary, push the ribbons into neat order with a brush and place the slide in a warm place to dry thoroughly. If the sections are required quickly it is possible to hasten the process by blotting the slide with several changes of filter paper soaked in absolute Alcohol.

Before such a slide can be stained, the wax must be removed. Plunge the slide into absolute Alcohol for a few minutes to harden the Albumen Fixative and transfer to Xylol, or Dioxane, which will dissolve the wax, leaving the sections adhering to the slide. Wash in 95 per cent. Alcohol. The preparation thus made may be stained on the slide by any of the standard methods. It should be noted, however, that the length of time required to stain microtome sections is much longer than that needed for hand sections, *e.g.*, 12 hours, because the former are so much thinner and require more stain to make them visible.

Remember that the Xylol used to wash off the wax must not be used for the final clearing before mounting in Balsam or a film of wax will be left which will spoil the preparation. The Balsam used for mounting such slides should be thinner than that employed for hand sections, and care must be taken to see that no air is left when the cover-glass is lowered on the sections.

Diagrammatic Scheme of the Staining Processes for Sections, as described in the Text. See pp. 10 to 26.


Fixing
Bouin's Fluid
Chrom-Acetic
Flemming's Solution
Water
25% Alcohol
Alcohol-Formalin Mixtures
Preserving
Material may be preserved in this
50% Alcohol
Hydrogen peroxide (for Flemming material)
Section Cutting
Staining
Water
Iron Alum
Heidenhain's Haematoxylin
25% Alcohol
Ehrlich's Haematoxylin
Eosine (or other simple stain)
Gentian Violet
Safranin
50% Alcohol
Acid Alcohol
Magenta and Methyl Green
Ammonia
Water
Iodine
20% Glycerine
Bismarck Brown
75% Alcohol
Safranin in 50% Alc.
Spirit Blue plus Picric Acid
95% Alcohol
50% Glycerine
Dehydrating
Absolute Alcohol
Erythrosine or Light Green in Clove Oil or Cellosolve
75% Glycerine
Clearing
n-Butyl Alcohol
Xylol-Alcohol 50 : 50
Clove Oil or Cellosolve
Clove Oil or Cellosolve
Xylol
100% Glycerine
Mounting
Canada Balsam
Euparal
Glycerine Jelly
Label
Label
Label

PART TWO

CYTOLOGY

Section 1. Elements of Cell Structure

A. THE MATURE CELL

1. CELL WALL

> ***Sambucus nigra*** (Elder).—Transverse section of pith from a young stem. Place the section in 95 per cent. Alcohol for a few minutes to remove the air from the cells, then transfer to water and mount in Glycerine.

Elder pith is one of the materials from which it is easiest to cut thin sections (see Part I, p. 14), and the beginner may well start with it and practise until he can cut sections which are uniformly not more than one cell thick before he attempts the study of plant anatomy under Part IV. Elder pith is also frequently used to hold and support small objects which it would be difficult otherwise to hold in the fingers while cutting sections of them.

The cells are empty when mature, so that the sections show only the **cell walls.** When examined with the low power the outlines of the cells resemble the bubbles in a foam, and if we allow for the fact that the wall of each cell is solid, not liquid, the analogy is quite a good one. Each cell is a closed " box " surrounded by its own wall of transparent colourless **cellulose.** As the cells are more or less rounded and similar in size, they do not fit very closely together and appear to overlap each other somewhat, but where any two cells are in close contact their walls are flattened by mutual pressure. As each cell has a wall of its own, the dividing wall between any two cells is necessarily *double*, and small **intercellular spaces** may be seen at the corners where three cells meet. Each wall will be seen to be studded over with numerous small rounded spots. These are thin places or **pits** in the wall. Under the high power examine one of the edgewise views of the walls which contain pits, and the latter will be seen as thin places closed only by a very delicate **pit membrane.** This membrane is really a portion of the common or " party " wall, which lies between the walls of adjacent cells, forming a third layer or **middle lamella** of the wall.

While looking over your sections with the low power, clusters of smaller cells will be seen, here and there, surrounding a cell with brownish contents. These are secretory cells and the brown material is tannin.

2. LIVING CELLS

> ***Allium cepa*** (Onion).—Cut an onion open and from one of the inner scales peel off a small piece of the epidermis from the outer surface. The membrane peeled off should only be one cell thick. Mount in Iodine.

The cells have thin cellulose walls, and every cell shows clearly a single **nucleus,** stained yellow. The **nuclear membrane** is easily made out, and the solid-looking **nucleoli** (one or more per nucleus). The structure of the nuclei themselves is obscure, but some at least will show granulations of **chromatin.** This, however, may be much better seen from permanent preparations stained with Haematoxylin (see Part I, p. 17).

Besides the nucleus, each cell contains **cytoplasm,** seen as a transparent granular matter, fixed by the Iodine, spread throughout the cell, but principally in a layer round the wall. Small rings may often be observed in it which are the outlines of **vacuoles.** The spaces not occupied by cytoplasm contain **cell sap,** and these, together with the smaller vacuoles, form the vacuolar system or **vacuome** of the cell.

Notice that the outline of each cell is convex, as befits an osmotically active sac in which there is an internal turgor pressure. In a tissue of this sort, where the cells fit tightly together, *the walls make no re-entrant angles.*

It is instructive to compare the above with a portion of the epidermis stripped from the inner surface of the same scale and mounted in water. The outline of the cells is similar, but here the cytoplasm forms a curious network of threads connecting the centrally placed nucleus to many points on the cell wall. In these strands of cytoplasm flowing movements may be seen, and they contain numerous minute shining granules, the **mitochondria,** which are constantly found in cytoplasm but about which comparatively little is known.

> ***Symphoricarpus racemosus*** (Snowberry).—Tease out a small portion of the soft tissue of the fresh berry with needles into a drop of water on a slide. Add a cover-glass and tap on it gently with the point of a needle to separate the cells from one another.

This **mesocarp** tissue is very loose and the large cells are not regular in shape as they are in most compact tissues, but are of the most varied and fantastic forms, touching one another, for the most part, only at a few projecting points.

The **cell walls** are thin, and the **cytoplasm** is unusually abundant. The **protoplasmic utricle** is quite thick and easily seen, and the **nucleus** may be also clearly seen, either embedded in the utricle or supported by protoplasmic anchoring strands. Notice the many fine colourless granules, the **mitochondria,** in the cytoplasm. They may be distinctively stained with a dilute, aqueous solution of Janus Green.

Treat the preparation with a little Iodine solution. The structure of the cytoplasm is greatly altered and becomes much more granular (coagulation), but the Iodine makes it more clearly visible in all its extent.

It is difficult to explain how turgid cells can be so irregular in form, when more or less isolated, as these are. Probably local differences in the composition and rigidity of the cell wall may account for it.

B. PROTOPLASM AND THE MERISTEMATIC CELL

1. CYCLOSIS

Elodea canadensis (Canadian Pond Weed).—Young leaf, from near the apex of the shoot, picked off carefully with needles, using as little force as possible. Mount in a drop of the same water in which the plant is living; *not* in distilled water.

The shock of detaching the leaf causes the protoplasmic movement to cease temporarily, but at room temperature it should begin again within five minutes.

The leaf is only two layers of cells in thickness, except at the midrib. The cells are rectangular and show fine **chloroplasts** (see Section 2, p. 39), some of which may be seen in the process of division. The cells which show **cyclosis** best are those long cells which lie immediately over the midrib. The **protoplasmic utricle** may be seen, with care, as a transparent stream flowing round the cell, but the chloroplasts make the most conspicuous features, as they are carried passively along in the cytoplasmic current. Their motion is relatively cumbrous and the cytoplasm moves considerably faster than they do, but their speed is roughly proportional to that of the utricle, so that they may be taken as an approximate index of its velocity. As an example, a chloroplast was timed to take thirty seconds at a temperature of 19° C. in running round a cell, a distance of 125 μ. This is equal to 1 mm. in four minutes, or an inch in an hour and forty minutes.

Tradescantia virginica (Spiderwort).—The handsome purple flowers of this species contain tufts of coloured hairs attached to the stamens. Some of these should be carefully detached with needles and mounted in water or in 0·7 per cent. Sodium chloride.

Each hair consists of a row of barrel-shaped cells attached end to end. Each cell contains a purple **anthocyanin** pigment, dissolved in the vacuole sap. This renders the colourless nucleus and cytoplasm conspicuous. After a few minutes streaming movements appear in the protoplasm, rendered visible by the motion of the minute granules. These movements do not form a regular circulation, but currents move in various directions, creating a complex network over the cell wall. Sometimes two opposite currents can be seen travelling simultaneously in the same minute thread of cytoplasm, as shown by the lively movements of the granules. The cell wall has a delicate spiral striation of its own, but this does not interfere with vision of the cell interior. The nuclei look practically structureless, the granular appearance which they show occasionally being due to the accumulation of cytoplasmic granules round them.

Cyclosis may also be observed in many root-hairs, *e.g.*, those of the Radish. Seeds should be germinated on moist blotting paper in a covered saucer and small portions of the first root, with the attached hairs, transferred to water on a microscope slide.

Other suitable objects for the observation of protoplasmic streaming are : hairs from the leaves of *Primula*, various species ; staminal hairs from the flowers of *Anagallis arvensis* (Scarlet Pimpernel) ; or hairs from inside the corolla of *Lamium album*, the White Dead Nettle. For experiments on Cyclosis, see Part VII, p. 381.

2. PROTOPLASMIC CONNECTIONS

Iris pseudacorus or ***I. foetidissima***.—*Thin* sections of the interior tissue (endosperm) of the ripe seed. Stain for two hours in Picro-aniline Blue (Soluble Blue). Wash in water. Mount in Glycerine. Rapid preparations may also be made by direct staining for a few minutes and mounting without washing. It is sometimes an advantage to treat the sections for two to three minutes with 30 per cent. Acetic acid to swell the tissues somewhat before staining.

Thick-walled seed endosperms are generally recommended for the observation of protoplasmic connections between cells on account of their large and prominent pits. Seeds of the *Iris* are among the best for this purpose. The stain colours the cytoplasm intensely, but leaves the walls uncoloured. Look for good sections through pits and examine them carefully with the highest magnification available. A 2 mm. oil immersion lens is advisable. The **protoplasmic threads** across the pit membrane may be seen as exceedingly thin blue lines crossing the membrane from side to side.

If an oil-immersion lens is used with a preparation mounted in Glycerine, it is desirable to avoid the drag of the oil on the cover-slip, and for this purpose one may substitute Anisole for the usual immersion oil. Its refractive index is the same, but it is non-viscous. Clean it off the lens as soon as the observations have finished.

Allium cepa (Onion).—Remove a *small* portion of the *outer* epidermis of one of the scales from near the centre of the bulb. Mount directly in 50 per cent. Sulphuric acid to which has been added a saturated solution of Soluble Blue (= Hoffmann's Blue of older authors) at the rate of 0·5 c.c. of the dye solution to 10 c.c. of acid. Take care that the tissue is properly wetted by the solution.

The protoplasts are fixed with practically no shrinkage, though the structure of the nucleus is destroyed. The cellulose part of the cell wall swells and becomes invisible, leaving the **middle lamella** free between the cells. The cytoplasm slowly picks up the blue stain. After the lapse of a few minutes examine the preparations carefully with the high power.

Numerous very fine strands of the cytoplasm may be seen connecting neighbouring protoplasts through the middle lamella. These threads pass through pits in the cell wall. The latter can easily be seen in untreated material mounted in water, but the threads are not visible without first causing the wall substance to swell with acid.

Aucuba japonica.—Sections of the endosperm from the berry still in the green state. Mount in 50 per cent. Sulphuric acid and Soluble Blue (see *Allium cepa*).

The cell walls show very clear and numerous **pits.** As the walls swell in the strong acid, look for a pit which is well seen in section and note that the rather sparse, granular cytoplasm may be seen entering the pit on both sides and meeting in the plane of the middle lamella.

3. PLASMOLYSIS

Beta vulgaris (Garden Beet).—Surface sections (or transverse sections) of the petiole of one of the varieties with red foliage. Mount in water.

The **epidermis** itself is colourless, but the cells of the **parenchyma tissue** below are all filled with a crimson anthocyan pigment. In water this colouring matter appears to fill the whole cell, the surrounding protoplasmic utricle being extremely thin. The nuclei may be seen, however, each surrounded by a small cluster of chloroplasts.

Now irrigate the preparation with strong (10 per cent.) cane sugar solution by drawing a drop under the cover-glass with a strip of blotting paper. The cell contents, including the colouring matter, will now be seen to have shrunk away from contact with the cell wall, owing to the withdrawal of water from the cell vacuoles, and the utricle may be seen as a delicate colourless membrane round the coloured sap. The nucleus is unchanged in appearance.

Now lift the cover-glass and remount the section in pure water. The cells rapidly de-plasmolyse and regain their original appearance, although there is a certain amount of leakage of colouring matter into the water. This escaping colour often stains the nuclei of the colourless cells.

Surface sections of the petiole of Rhubarb may be used instead of Beet if they are similarly red-coloured.

Plasmolysis may be utilized as a means for measuring the osmotic pressure in cells. This is dealt with in Part VII, p. 306.

4. MERISTEMS

Vicia faba (Broad Bean).—Longitudinal (or transverse) sections, cut through the tip of a young seedling root, not more than a few millimetres from the apex. Stain for a few minutes in Methyl Green-Acetic acid and wash in water. Counterstain with Erythrosine, Acid Magenta or saturated aqueous Picric acid.

The **cells** are more or less cubical and are packed together with no intercellular spaces. The walls are gelatinous and the cytoplasm and nucleus completely fill the interior with few or no vacuoles present. The development of vacuoles is indeed the first sign that the meristematic phase of a cell's life is over. The nuclei are exceptionally large for the cells, and the structure is easily seen. Division figures are generally visible (see p. 37), especially in longitudinal sections. This feature gives the name **meristem** to these young tissues, from the Greek work "meristos," meaning "divisible." There are usually four zones of cells present (Hanstein's layers) :—

THE DERMATOGEN.—An outer layer, one or two cells in thickness. The cells are elongate (in longitudinal view) and have sparse contents. This forms the **piliferous layer** and later the **exodermis.**

THE PERIBLEM.—An intermediate zone of squarish (longitudinally) or circular (transversely) cells, with dense cytoplasm and a large nucleus which nearly fills the cell. This forms chiefly the **cortex** of the older root.

THE PLEROME.—A central core of narrow, longitudinally elongated cells, which mostly contribute to the formation of the **vascular tissues.**

It may often be seen in longitudinal sections that the meristematic cells form groups or packets, four or five together longitudinally, which have a common investing wall and are the product of division of a single parent cell.

THE CALYPTROGEN.—An apical layer producing the **root cap**.

C. NUCLEAR DIVISION

Vicia faba (Broad Bean).—Mitosis in the apical meristem.

The following observations can only be made upon properly fixed and stained material. Good microtome sections for the purpose are easily purchased if necessary. Other roots may be substituted for *Vicia*, *e.g.*, Onion, Maize, Pea, *Tradescantia*, etc., as all show the same general appearances (Fig. 4). The principal stages of mitosis are enumerated below :—

(*a*) Nucleus with **nuclear membrane** and with **chromosomes** in process of formation, but irregularly distributed all over the nucleus and often contorted. The **nucleolus** is usually visible. This is the **prophase.**

In the earliest stages of the prophase the chromosomes form thin, contorted bands, often with a suggestion of spiral coiling. These bands thicken and shorten, finally becoming the separate chromosomes. This is the typical late prophase appearance described above.

(*b*) Nuclear membrane disappears. Chromosomes arranged in a loose longitudinal bundle. Early **metaphase.**

(*c*) Chromosomes showing a longitudinal split and bending so as to arrange themselves across the middle of the cell. **Metaphase.**

(*d*) Chromosomes longitudinally divided and arranged transversely across the cell (**equatorial plate**), their outer ends bent out forming two groups pointing towards the opposite ends of the cell. Late **metaphase.**

(*e*) Daughter-chromosomes separating into two groups, converging towards the two ends of the cell (**diaster stage**). At this stage the separation

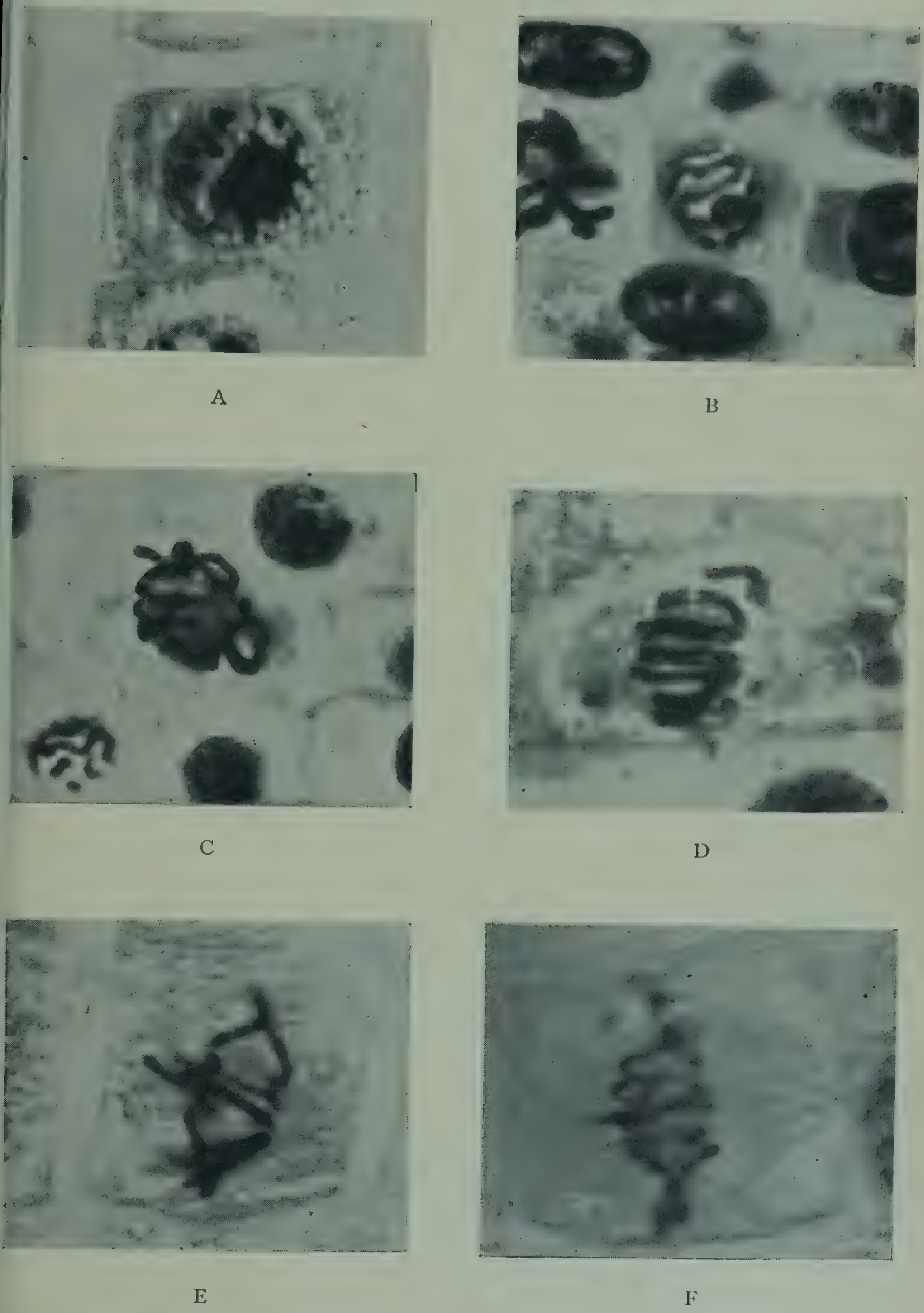


FIG. 4.—Stages of mitosis in the root of *Vicia faba*. A, Metabolic nucleus. B-D, Stages in prophase. E-F, Metaphase.

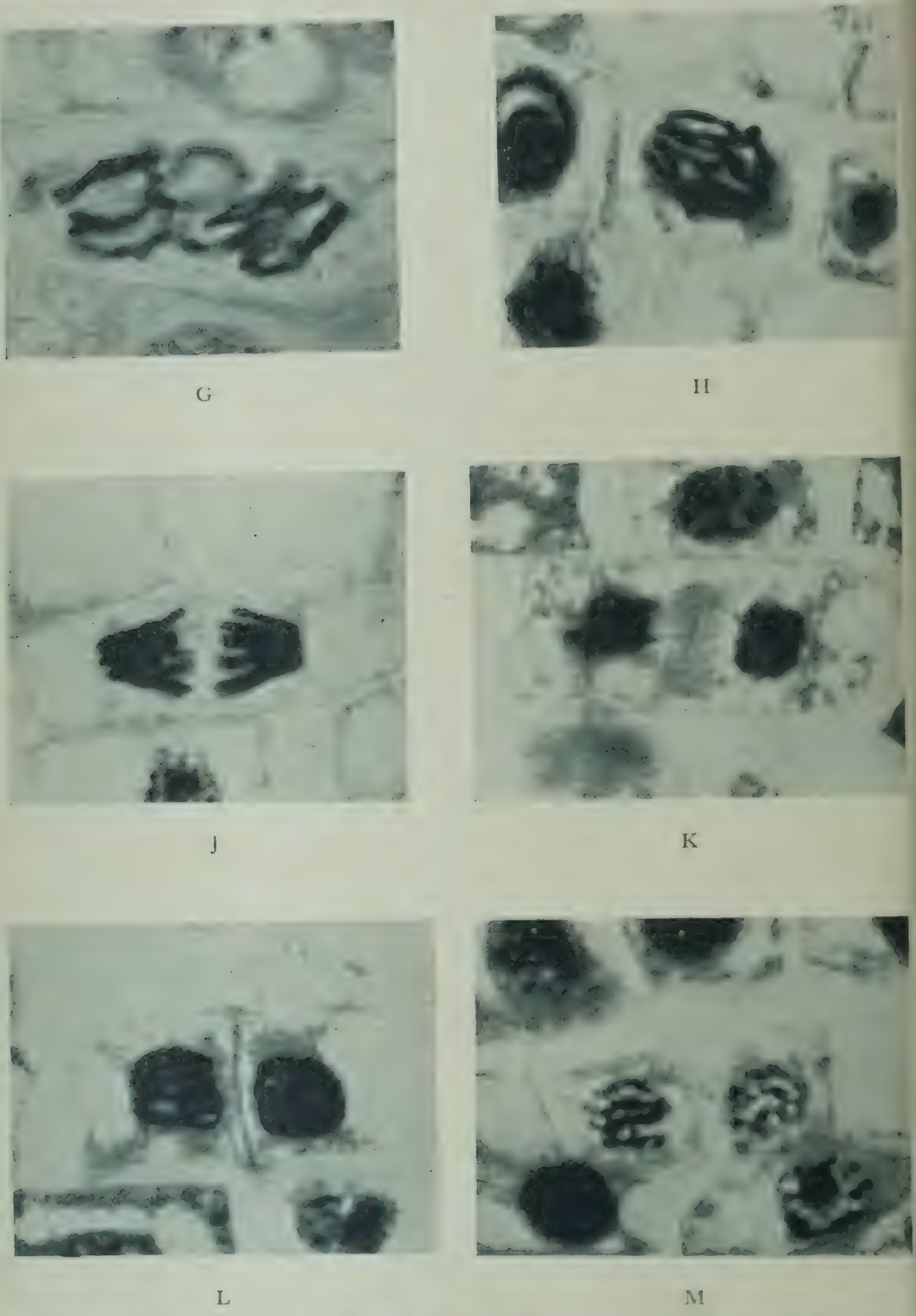


Fig. 4.—Stages of mitosis in the root of *Vicia faba*. G-J, Anaphase. K-M, Telophase and stages in the formation of the cell-plate and reconstitution of the daughter nuclei.

of the two halves of each chromosome is complete. In larger cells the **achromatic spindle fibres** may be seen connected to the convergent ends of each conical group of chromosomes. **Anaphase.** This is a very characteristic and easily recognizable stage.

(*f*) Chromosomes in two distinct clusters, the individuals thick, short, and closely packed together. Achromatic spindle fibres visible *between* the two groups, with the beginnings of a cell-plate, *i.e.*, a new wall, forming transversely across the cell. **Telophase.**

(*g*) Chromosomes no longer visible, being closely balled together into two deeply stained masses, the two new nuclei. Nuclear membranes reformed. Cell-plate complete, dividing the cell into two portions. Late **telophase.**

Appearances which cannot be fitted into the foregoing scheme are probably from fragmented nuclei, and may be ignored at this stage.

Section 2. Cell Inclusions and Reserves

A. CHLOROPLASTS

Mnium hornum or ***Funaria hygrometrica***—Leaf mounted in water.

Part, at least, of the moss leaf is only one cell in thickness and consequently the cells, which are large, show the chloroplasts very plainly. The plastids are more or less circular in face view, but flattened in profile. They multiply by fission, and hour-glass forms, which are frequently seen, represent stages in this process.

Under the high power it will be seen that the plastids are not homogeneous, but that the chlorophyll is dispersed as minute specks in a colourless matrix, giving the plastid a granular appearance. There is no visible membrane round the plastid, as there is around the nucleus, but fine cytoplasmic threads may be seen linking them to each other.

Another moss, *Catharinea undulata*, is very favourable material for the examination of chloroplasts. Sections of the thallus of the common Liverwort *Fegatella conica* show very fine chloroplasts which have the peculiarity of containing large starch grains, like those usually found in storage organs.

Elodea canadensis and other higher plants.—The chloroplasts of higher plants may be well studied in the leaf of *Elodea canadensis* (see under Cyclosis, p. 33). Sections of the leaf of the Garden Geranium (*Pelargonium zonale*) or of Privet (*Ligustrum vulgare*) also make suitable material. The minute grains of **starch** included in the body of the plastid can usually be seen without special treatment,[1] but the starchy nature of these bodies is best demonstrated by mounting sections, or whole young leaves of *Elodea*, in a saturated solution of Chloral hydrate coloured by the addition of Iodine solution. This causes swelling and blue coloration of the minute starch grains and decolorizes the chlorophyll.

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[1] *Euonymus japonica* and *Cochlearia anglica* and the fleshy leaves of certain other plants show large storage starch grains in the green leaf tissue, which is unusual.

The chlorophyll may also be bleached with a solution made by dropping strong Hydrochloric acid on crystals of Potassium chlorate. When a green colour shows that Chlorine is set free, add 50 to 100 c.c. of 60 per cent. Alcohol. After bleaching in this solution the starch grains may be stained in an ordinary Iodine solution.

B. CHROMOPLASTS

Tamus communis (Black Bryony).—Cut epidermal sections of the berry.

In the green state the cells contain very small round chloroplasts, but in the ripe red berry these have been transformed into rod-like **chromoplasts** of a beautiful orange colour, due to **carotin** ($C_{40} H_{56}$).

Lycopersicum esculentum (Tomato).—Scrape a little of the sub-epidermal pulp from a fruit which is perfectly fresh and recently ripened and mount in water or in 0·7 per cent. salt solution.

Each cell contains a large number of orange-coloured **chromoplasts.** In old fruits these become clumped together and disintegrated, though their colouring matter remains unchanged.

Rosa sp. (Rose, any species).—Sections through the fleshy tissue of the fruit (the " hip "). Mount in Glycerine. As in the case of the Tomato, the material should not be too old.

The colour in the surface layer of the hip is a sap pigment, but in the underlying tissue the colour is due to the orange-yellow plastids in the cells. These have not the regular appearance of chloroplasts, but assume a great variety of shapes and sizes, many of them angular, as if their substance were crystalline. The colouring matter is **carotin.**

Daucus carota (Carrot).—Sections of the tap-root mounted in Glycerine.

The chromoplasts are orange-coloured and contain carotin instead of chlorophyll. Most of them are definitely crystalline in outline, the pigment being apparently held in crystalline form.

C. ANTHOCYANINS

Pelargonium zonale or other garden species of *Pelargonium* (Garden Geranium).—Transverse section of the petal.

The section shows a colourless interior tissue, with an upper and lower epidermis of bluntly conical cells. These cells contain the red colouring matter (**pelargonidin**) in solution, filling the cell vacuoles. There are no plastids present.

The pigment is an acid-alkali indicator, like litmus. Remove the cover-glass and invert the slide over the mouth of a bottle of Ammonia. The colour turns to a beautiful purple-blue.

Petals of red roses may be used instead of *Pelargonium.*

Lamium album (White Dead Nettle).—Portion of anther wall, mounted in water.

The epidermis of the anther walls of *Lamium album* has nuclei which are stained deep blue by the anthocyanin present in the cell sap, which is purplish in the vacuoles. The colour of the nucleus persists after treatment with Iodine, which precipitates the sap pigment as a greenish substance.

D. LEUCOPLASTS

Lathraea squamaria (Toothwort).—Sections of the fleshy underground leaves should be stained for some hours in 0·2 per cent. Acid Fuchsine (Magenta or Rubin), washed in water and mounted in Glycerine.

The starch grains in the leaves of this parasite show the **leucoplasts** plainly, each stained pink. They appear like little excrescences, usually at one end of the oval starch grains. In favourable cases a delicate pink-stained membrane may be seen stretching from the leucoplast entirely round the grain, which is thus actually enclosed within the greatly distended body of the plastid. See also page 42 for observations on the starch grains.

Ranunculus ficaria (Lesser Celandine).

The tubers of this plant contain large starch grains, many of which show small leucoplasts attached, like little excrescences, to the starch grains. The younger the tubers the better for this purpose. The leucoplasts do not stain readily with Acid Fuchsine.

Parietaria diffusa (Pellitory).—A transverse section of the stem stained with Acid Fuchsine and well washed with water will show the leucoplasts attached to the starch grains in the xylem and cortical parenchyma cells.

If material is available, the cells of the stem cortex in *Pellionia*, or of the pseudo-bulb of the orchid *Phajus* show extremely good leucoplasts.

E. STARCH GRAINS

Solanum tuberosum (Potato).—Scrape a little material from a cut surface into a drop of dilute Iodine and Glycerine mixed, on a slide; or else cut a very thin section and mount in the above mixture.

The grains are large, irregular in shape and size, and occur packed together in the cells. Most are simple, *i.e.*, they have a single **hilum** or growth centre, round which the grain grows in a series of **excentric layers,** which may be seen by closing the iris diaphragm of the sub-stage condenser, or by swinging the mirror aside to give oblique lighting, if there is no condenser on the microscope. The preparation has a most striking appearance under darkground illumination (Part I, p. 7). See also observations with polarized light under Microchemical Tests (Part II, p. 49). With Iodine, Potato starch does not give a pure blue colour, but various tints between purple and blue, due to admixtures of Dextrine with true starch in the grains.

> ***Lathraea squamaria*** (Toothwort).—If material is available for the observation of leucoplasts (p. 41) mount additional sections in Iodine and observe the starch grains.

The growth centre or **hilum** is central, and there is **concentric lamellation** of the grains, which are exceptionally large and regular in shape.

> ***Triticum vulgare*** (Wheat).—Corrosion of starch. Take a seedling of Wheat about 2 in. high, with the grain still attached. Cut open the grain and squeeze out a drop of the milky contents on to a slide. Add a drop of Glycerine and a drop of Iodine solution. Mix and cover with a cover-slip.

Under the high power the starch grains will be seen to show a purplish colour, not the typical blue-black, and they show various stages of corrosion under the influence of diastase. The first stage is usually an irregular crack at the hilum. From this branched channels run out to the edges, splitting the grain into fragments. Channels also run round the striation layers of the grain forming circles, while irregular pitting occurs. Eventually the whole starch grain is hydrolysed to Maltose and disappears.

F. INULIN

> ***Dahlia variabilis***.—Sections of the tubers, which have been previously steeped for some days in 95 per cent. Alcohol. Mount in Glycerine. (See also Part II, p. 49.)

The tissues consist chiefly of parenchyma cells, all of more or less equal size and with thin walls. Here and there will be seen (particularly around vascular bundles) large rugged masses, with rounded outlines, formed of **inulin,** which is in solution in the cell sap during life, but is precipitated by strong Alcohol, in which it is insoluble.

> ***Taraxacum officinale*** (Dandelion).—Longitudinal sections of portions of tap root which has been kept for some days in strong Alcohol.

These show large, irregular crystalline masses of Inulin in the tissues. They are quite unmistakable, even under the low power.

G. CELL CRYSTALS

Scilla non-scripta (Bluebell) or ***Hyacinthus orientalis*** (Garden Hyacinth).—Sections of the bulb scales mounted in Glycerine.

Many bundles of fine needle-shaped crystals of **calcium oxalate,** called **raphides,** may be seen in the tissues. Each needle is double-pointed and is probably a slender prism with a very acute pyramid at each end (*cf. Tritonia* crystals). The needles lie in parallel bundles in large cells and are surrounded by mucilage.

Many other Monocotyledons show raphides, *e.g.*, *Cordyline australis*, where they are exceptionally large.

Lemna minor (Duckweed).—Soak one of the young fronds in strong Alcohol until decolorized. Transfer to absolute Alcohol for a few minutes and then to Clove Oil. Mount in the latter.

The transparent frond will be seen to contain numerous bundles of **raphides,** arranged with the longest in the middle of each sheaf. The large cells in which the bundles are formed may be clearly seen. Polarized light shows up the distribution of the crystals very beautifully under the low power.

Stain the frond with Corallin dissolved in 4 per cent. Sodium carbonate and then wash out with water. This stains the mucilage specifically and enables the mucilage round each bundle of crystals to be distinguished clearly.

Circaea lutetiana (Enchanter's Nightshade).—Leaves similarly treated show good raphides.

Among other good material for raphides may be mentioned the leaf or petiole of Rhubarb, and epidermal sections from the berry of *Tamus communis* (Black Bryony) (see under Chromoplasts, p. 40). The phloem of *Vitis inconstans* (Virginia Creeper) shows the enclosing cell and dense mucilage particularly well, even without staining.

Tritonia (Montbretia) crocosmiaeflora (Garden Montbretia).—Sections of the corm tissue, taken in any direction.

There are extremely large crystals of **calcium oxalate** embedded in the tissues, easily seen with the low power, or even with a hand lens. They are long four-sided prisms, with a pyramid at each end, really a development of a four-sided double pyramid like that seen in *Begonia* and in many other plants. Each individual crystal lies in a specially large cell with a highly thickened, lamellose wall which surrounds the crystal. In the petiole of *Begonia*, described under Collenchyma, page 51, there are very good octahedral (double pyramid) crystals of **calcium oxalate** in the parenchyma cells of the ground tissue. They are easily seen under the low power.

Some of the crystals show twinning, *e.g.*, the intergrowth of two or more crystals together.

Myriophyllum spicatum or other species (Water Milfoil).—Transverse section of the stem internode.

The cortex shows large intercellular spaces or **lacunae** (see p. 387) on the walls of which are **cluster crystals** of Calcium oxalate. Each aggregate is spherical and consists of a number of octahedra, *i.e.*, double pyramids, radiating from a common centre, their points extending in all directions. Each aggregate is formed in a special cell, the wall of which may be seen (stained with Eosine, if necessary) as a delicate sac surrounding the crystals.[1]

Parietaria diffusa (Pellitory).—Transverse sections of the leaves.

The upper epidermis is formed of large colourless cells over a well-developed palisade. The **cystoliths** are very numerous, formed in large special cells or **idioblasts** extending downwards from the upper epidermis into the green mesophyll. Each cystolith stands out prominently, due to its relatively high refractive index, and is covered with pointed wart-like projections. It consists of **Calcium carbonate**.

Lift the cover-glass and add a drop of dilute Hydrochloric acid. The cystolith dissolves easily, producing bubbles of Carbon dioxide. When solution is finished, observe that there remains in each cystolith cell a small pointed peg of cellulose projecting downwards from the top of the cavity of the cell. This peg formed the nucleus of the cystolith, and upon it the deposition of mineral matter began.

Many other members of the Nettle family (*Urticaceae*) will also show cystoliths. Those in the leaves of the common stinging nettle are particularly large, but irregular in shape.

Ficus elastica (India-rubber Plant).—Transverse sections of the leaf.

The upper epidermis is multiple, *i.e.*, consists of three layers of cells; one the true **epidermis,** of small cells with very thick cuticle, below which are two layers of large **hypodermal** cells. The upper palisade layer is very well developed, and there is even a short, lower palisade layer against the lower epidermis. Such anatomical characters are common among tropical sun plants.

Here and there a cell of the hypodermis is much enlarged and penetrates downwards into the palisade layer. From the upper end of this cell, the **idioblast,** there grows downwards into its lumen a **cellulose peg,** on which accumulates an irregular crystalline mass of **Calcium carbonate,** the **cystolith.** This dissolves in acids, with the evolution of Carbon dioxide leaving the cellulose peg visible. The latter is club-shaped and shows a lamellated structure when stained.

[1] Sections, or small portions of leaves containing crystals may be cleared in Eau de Javelle or in Clove Oil (after dehydration) and examined in polarized light. The crystals show up remarkably well and make a striking demonstration.

H. ALEURONE GRAINS

Ricinus communis (Castor Oil Plant).—Thin sections of the endosperm of the seed, mounted in Olive Oil or Castor Oil.

The cells contain numerous **aleurone grains,** which are rounded bodies, each with a delicate membrane of its own, enclosing a hexagonal crystalline body, the **crystalloid,** which is a protein, and a smaller round body, the **globoid,** with a relatively high refractive index (*i.e.*, it stands out brightly), which consists of a double phosphate of Calcium and Magnesium.

These compound grains are a special form of reserve food vacuole sometimes found in oily seeds.

If mounted in aqueous fluids the crystalloid swells and loses its characteristic outline.

Sections may also be stained in Nile Blue sulphate, as under *Bertholletia* in the following paragraph.

Bertholletia excelsa (Brazil Nut).—Thin sections of the endosperm may be stained in a solution of Nile Blue in 10 per cent. Sulphuric acid, which has been boiled for about half an hour. Stain for a few minutes, wash well in water and mount in Glycerine.

The **aleurone grains** stain blue, the **oil drops** pink. It may be seen that each grain has its own external membrane. Several protein granules may often be seen in one aleurone grain, but they have not the crystalline outline of those of *Ricinus.*

Triticum vulgare (Wheat).—Transverse sections of the wheat grain, stained with Iodine and mounted in Glycerine (see also p. 49).

Aleurone grains have not always the complex form seen in *Ricinus* or *Bertholletia.* The central part of the Wheat grain contains only starch as a reserve, but there is a layer of cubical cells, just under the protective coat of the grain, which stains yellow with Iodine and contains a dense abundance of protein grains. This is known as the **aleurone layer.** This layer is removed by modern processes of milling (except for brown flour), as it makes the flour yellowish and impairs its keeping qualities. The protein nutriment is, of course, lost to the flour by this procedure.

Section 3. Microchemical Reactions

The different substances of which the cell walls in plants are made up may be recognized by **microchemical tests,** that is to say, by reactions yielding colours or precipitates, which can be recognized microscopically. The known number of these reactions is not great, and the discovery of a new one is always valuable. The following tests are standard ones and should be carried out for the identification of the more important forms of cell wall substance.

Formulae for the reagents needed will be found in Appendix I at the end of the book.

A. CONSTRUCTIONAL MATERIALS

Cellulose.—Pure filter paper (not cotton wool or wood wool, which do not react well). Sections of Elder pith or other pithy tissues.

(*a*) Soak for a few moments in Iodine; mount in a drop of 50 per cent. Sulphuric acid. Blue coloration.
(*b*) Mount in a drop of Chloriodide of Zinc. Blue-violet coloration.
(*c*) Soak in Schweitzer's reagent. Swells, gelatinizes and finally dissolves.

The walls of parenchymatous cells, although mainly consisting of cellulose, often contain fatty acids, etc., which may hinder the cellulose reactions unless they are previously removed by treatment with Potassium hydroxide solution.

Hemicelluloses (Polyuronides).—Sections of Iris seed or of Date stone, previously softened by soaking in 50 per cent. Glycerine. No general microchemical tests available.

Mount in dilute Iodine. Blue coloration (amyloid). This reaction is not given by all hemicelluloses. Amyloid is not a true polyuronide but a hydrated cellulose.

Lignin.—Wood shavings or sections of any sort of wood, *e.g.*, match sticks.

(*a*) Aniline chloride or sulphate. A bright yellow coloration. It will be found in sections that xylem gives a somewhat greenish colour, while sclerenchyma gives a true bright yellow colour.
(*b*) Phloroglucin and Hydrochloric acid. A cherry-red coloration.
(*c*) Reduced Fuchsin. Deep red coloration. This is known as Schiffs' test. It is given by all compounds containing free aldehydes.

Suberin.—Sections of bottle cork or of natural bark.

(*a*) Stain with Sudan III[1] and wash in 50 per cent. Alcohol. Mount in Glycerine. Only the suberized walls retain the red stain. The reaction is due to the presence of fatty acids and soaps.
(*b*) Stain in a freshly prepared alcoholic solution of chlorophyll. Suberized walls and cuticle maybe stained green.
(*c*) Alkannin tincture (see p. 50). Deep red coloration. If the section contains woody tissue soak for two or three days beforehand in Eau de Javelle. This destroys the staining quality of the lignified cells, but not that of the suberin.

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[1] Sudan IV (Scharlach Red) can also be used.

Cuticle.—Transverse sections of the leaf of *Yucca gloriosa*. *Ilex aquifolium* (Holly) and *Agave* spp. are also good. Stain with Sudan III and wash in water. Mount in Glycerine.

Both sides of the section show an **epidermis,** of one layer of cells, covered externally by a thick layer of **cuticle,** which is stained pink. This forms a continuous sheet outside the epidermis. Round the stomata it is turned up into a conspicuous ridge. The cuticle is composed of fatty acids. It is waterproof, and its thickness is more or less directly related to the dryness of the conditions under which the plant usually grows.

Mucilage.—Sections through the dry testa of Linseed, of Wallflower or of *Collomia coccinea* seeds. In the latter the mucilage is in the form of a coiled spiral in the epidermal cells, which is everted, and elongates itself in a remarkable fashion when sections of the dry seed are mounted in water. Sections of many monocotyledonous stems and leaves, especially of those of bulbous plants, also make good material.

(*a*) Stain in Corallin-soda and wash in water. Mount in Glycerine. The mucilage is stained bright pink.

(*b*) Soak in 10 per cent. Copper sulphate, wash in water and transfer to 10 per cent. Potassium hydroxide. Mucilage is stained bright blue.

Pectic Substances.—Sections of any parenchymatous tissue, especially cortex of stems or roots.

Pectin is a constituent of most cell walls and occurs in three forms: Pectin (soluble), Protopectin (insoluble) and Pectic acid or its Calcium salt (insoluble). The middle lamella is chiefly composed of an insoluble pectin substance, probably protopectin.

Ruthenium Red (Ruthenium sesquioxide) stains the middle lamella dark red.

Callose.—Longitudinal sections through the phloem of *Cucurbita*, *Vitis* or *Tilia*, preferably in autumn. The Callose plugs are laid down as thick wads on each side of the sieve plates.

(*a*) Corallin-soda. Callose stains pink, like mucilage.

(*b*) Chloriodide of Zinc. Stains brown, especially if an equal quantity of Iodine solution is added to the reagent (Russow's reagent).

Silica.—Many cell walls are impregnated with colloidal silica. Use epidermal sections from the leaf of a Bamboo, *Ammophila arenaria* (Marram Grass) or other stiff grass.

Sections are soaked in 50 per cent. Nitric acid, washed, then dried on a piece of Platinum foil. This is heated to bright red heat in a Bunsen burner until only the ash remains. Cool and treat with a drop of dilute Hydrochloric acid and examine by reflected light on the foil, or else carefully invert on to a glass slide and mount in Hydrochloric acid. There is so much Silica in the cell wall that the epidermis remains coherent and forms what is called a **spodogram** of the tissues. Note, especially, short cells entirely filled with Silica.

B. CELL CONTENTS

Proteins are not only laid down as reserves in Aleurone Grains (see p. 45), but may occur free in crystalline form in the cell, particularly in the nucleus. The best reactions for their identification are as follows : –

(*a*) XANTHOPROTEIC TEST.—Treat a section, or a suspension of the tissue in water, with strong Nitric acid. A yellow colour is produced. Now add a few drops of strong Ammonium hydroxide. The colour changes to orange.

(*b*) BIURET TEST.—Add to the test solution 1 c.c. of 20 per cent. Sodium hydroxide and one drop of a 1 per cent. Copper sulphate solution. A violet colour indicates the presence of protein.

(*c*) MILLON'S TEST.—Millon's reagent (see Appendix, p. 460) gives a reddish colour with proteins, but it is of little value microscopically as the colour is difficult to see in the cells.

Fatty Oils.—Sections of the seed of Almond or of Soya Bean. Stain for a few minutes in Sudan III in 70 per cent. Alcohol, wash thoroughly in water and mount in Glycerine.

In the Almond the oil is present in large drops which stain red and may be seen both in the cells and floating round the section. In Soya Bean the oil is in very minute droplets, scattered in large numbers in each cell, giving the whole section an orange tint under the low power. Under the high power the individual deeply stained droplets may be seen.

Tannin.—Transverse sections of a young stem of *Fagus sylvatica* (Beech).—Place small pieces of the tissue, or sections, in saturated aqueous (7 per cent.) Copper acetate for at least ten days. Sections of the tissues are then laid in a 0·5 per cent. solution of Ferric acetate for a few minutes, washed in water, and mounted in Glycerine. Tannins colour either green or blue under this treatment, and in *Fagus* those in the bark colour green, while those in the pith show blue.

Resin.—Small shavings of Pine wood rich in resin are placed for a week in a saturated (7 per cent.) solution of Copper acetate. At the end of this time the sections are removed, washed in water, and mounted in Glycerine. Resin in the cells will be stained an intense emerald green.

The same coloration is shown by the aromatic " oils " (terpenes) which are common in plant glands and are closely related to the resins.

There are no really specific reactions for these substances which will separate them clearly from the tannins, for example. The above reaction should only be taken in conjunction with solubility in Alcohol, Ether or Chloroform, as a proof of the resin nature of a deposit.

Inulin.—Sections of roots of either *Dahlia variabilis* (Garden Dahlia) or *Taraxacum officinale* (Dandelion) are mounted in a drop of concentrated alcoholic solution of Orcin and a drop of concentrated Sulphuric acid is then added.

The Inulin sphaerites dissolve gradually and an orange colour appears in the sections, especially in the latex tubes of the Dandelion. Gentle warming increases the intensity of the colour.

Starch.—Sections of tubers of *Solanum tuberosum* (Potato) or of grains of *Triticum vulgare* (Wheat).

(*a*) Iodine solution ; dark blue-black colour. If the Iodine solution be diluted, a less intense bluish-purple colour is produced.

(*b*) Starch grains should be examined, if possible, with polarized light. Between crossed Nicol prisms, the starch, in common with the cell wall substance, shows marked bi-refringence and therefore appears light on a dark ground. In addition, however, a black cross with spreading arms will be seen, the centre of which is at the hilum of the starch grain. This is clear evidence of the crystalline nature of starch, for the black cross is the characteristic interference figure of monaxial crystals.

It may be noted that this bi-refringence of starch and cellulose is often useful in determining the plant nature of minute tissue fragments in pathology, medico-legal investigations, etc.

(*c*) Starch grains will also stain a mauve colour in dilute, aqueous Crystal Violet.

Sugars occur as reserve substances in many Monocotyledons and also notably in Carrot and Beet among Dicotyledons. Where sugar is formed as a reserve, starch is not produced. The sugar stored may be either Glucose or Sucrose.

(*a*) FEHLING'S TEST.—Boil a little chopped tissue of the underground storage organ of the Carrot in water in a test-tube and add equal quantities of Fehling's solutions I and II. Boil again. An orange-red precipitate of Cuprous oxide indicates the presence of a reducing sugar. Sugar-beet, which contains Sucrose, does not give this reaction.

(*b*) α-NAPHTHOL TEST.—To a water extract of the Sugar-beet, which contains Sucrose, add a little α-Naphthol solution, which is a 1 per cent. solution in 70 per cent. Alcohol. Run a little strong Sulphuric acid down the side of the tube. A violet ring is formed at the junction of the two liquids.

(*c*) HYDROLYSIS OF SUCROSE. To a fresh extract of Sugar-beet add a little Sulphuric acid and boil gently. Then add Fehling's solution. An orange-red precipitate shows that the Sucrose present has been hydrolyzed to Glucose and Fructose which are both reducing sugars. Ordinary Beetroot may be substituted for Sugar-beet in these tests.

Latex. Longitudinal sections of roots of *Taraxacum officinale* (Dandelion).

(*a*) An alcoholic extract of Alkanet root [1] (Tincture of Alkannin), which must be freshly prepared, gives a purplish-red colour with the contents of the latex tubes.

(*b*) Raspail's reagent. A saturated aqueous solution of Sucrose and concentrated Sulphuric acid, in equal quantities, freshly mixed, gives a pinkish-purple colour with latex, partly due, probably, to the caoutchouc granules in the latex emulsion.

Calcium Oxalate.—Sections through the corm of *Tritonia crocosmiaeflora*. (See under Crystals, p. 43.)

Mount the section in a drop of strong (about 80 per cent.) Sulphuric acid. After a short time delicate sheaves of needle crystals of Calcium sulphate will be seen growing out from the large oxalate crystals, due to double decomposition with the Sulphuric acid.

Section 4. Types of Mature Cells and Elementary Tissues

A. PARENCHYMA

Sambucus nigra (Elder). -Transverse sections of pith. (See also Part II, p. 31.)

The pith consists entirely of **parenchyma cells** which, in the fully grown stem, are dead and empty of protoplasm. The cell walls which remain form the tissue and show the characteristic isodiametric parenchyma shape with rounded angles and flattened sides which are produced by the mutual pressure on all sides of spheres with soft walls, just as in the analogous case of bubbles in a foam.

With the high power observe the small **intercellular spaces,** best seen at the angles of the cell walls and the **pits,** which look like small gaps in the walls. Close observation will show, however, that each pit is closed by a very delicate membrane, the **pit membrane,** which is part of the **middle lamella** of the wall.

Phaseolus multiflorus (Scarlet Runner Bean). Transverse sections of the cotyledons in the half-ripe state.

The cells contain numerous colourless leucoplasts. Stain with Iodine. A blue-black colour in the body of each leucoplast shows that starch formation is in progress. The leucoplasts produce the starch grains which are found in the cells of the ripe seed.

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[1] The dried root may be purchased from a druggist.

The cotyledons of many other large seeds may be used instead of Bean.

B. COLLENCHYMA

Tussilago farfara (Coltsfoot).—Transverse section through the petiole, stained in Aniline chloride and mounted in Glycerine.

The section is oval in outline, flattened at one side and consists mainly of **parenchyma** with small intercellular spaces, easily seen. There is also a horseshoe-shaped row of **vascular bundles.**

Externally, there is a single layer of **epidermal cells,** beneath which are three or four layers of cells with whitish masses of thickening material at the angles of their walls. This is **collenchyma** tissue, peculiar in the irregular character of its thickening, which is, moreover, of cellulose and not of lignin.

Begonia rex (or other species).—Transverse section of the petiole stained in Aniline chloride and mounted in Glycerine.

The **collenchyma** forms a layer, two to three cells deep, under the **epidermis.** These cells are parenchymatous in shape, like those of *Tussilago*, but there are conspicuous triangular masses of **cellulose thickening** at the angles of each cell.

See also under Crystals, p. 43.

Beta vulgaris (Garden Beet) also shows excellent collenchyma in the petiole, rendered specially conspicuous by the coloured cell sap.

C. SCLEREIDS

Pyrus communis (Pear).—Sections of the flesh of the fruit, near the core, stained with Aniline chloride and mounted in Glycerine.

The greater part of the tissue consists of large, thin-walled **parenchyma,** but scattered through this are groups of very highly thickened, lignified cells, the **sclereids,** or " stone cells." They stain intensely yellow with Aniline chloride. Each cell shows very clearly the system of narrow **branching pits,** looking like minute canals, which penetrate the lignified substance of the cell walls. In the wild Pear a much larger proportion of the fleshy part is made up of these hard woody cells.

Camellia japonica (Garden Camellia).—Transverse section of the leaf stained in Aniline chloride and mounted in Glycerine.

The leaf itself is somewhat leathery and has a highly cuticularized epidermis. The general sclerophyllous leaf structure is well shown. Note also the conspicuously granular appearance of the chloroplasts and the cluster crystals of Calcium oxalate (see Part II, p. 44). Here and there,

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especially near the midrib, there are very large lignified cells which lie across the mesophyll, nearly from one epidermis to the other. These cells, the **sclereids,** are highly thickened and of very irregular outline, showing branches and protuberances in various directions. The walls contain branching pits, like those of the sclereids of the Pear.

These sclereids are generally interpreted as mechanical supports for the leaf tissues, but this is only guesswork.

If available, the leaves of *Hakea pectinata* will also show excellent sclereids. Alternatively Tea leaves may also be used, if first soaked out in hot water and subsequently hardened in Alcohol before section cutting. The Tea plant belongs to the genus *Camellia.*

D. SCLERENCHYMA

> ***Rosa sp.;*** any garden or wild species will do. Transverse sections of the hard wall of an achene from inside the " hip." Cut the achene transversely in half and take sections across the cut surface. The tissue is very hard, but by gripping the achene in hardened Carrot or *Laminaria* stipe, satisfactory sections may be obtained without much difficulty. If necessary, the achene may first be boiled for a few minutes to soften it. Stain with Aniline chloride and mount in Glycerine.

The tissue is made up of very thick-walled cells, the **sclerenchyma,** some cut transversely, others longitudinally. The transverse sections show rounded outlines with lignified walls which are perforated by numerous narrow **pits,** like canals in the thickness of the walls. The wall substance usually shows **concentric lamellation.**

Longitudinal views may also be seen in the same sections, forming the inner layer of the achene wall (the pericarp). In this aspect the sclerenchyma cells appear elongated, narrow and pointed at both ends, *i.e.*, spindle-shaped, the pits appearing as numerous dark dots on the walls. The cells fit very closely together, making a very dense tissue.

Similar hard tissue from the fruit of the Hawthorn may be used as an alternative.

E. STELAR ELEMENTS (MACERATED)

> ***Apium graveolens*** (Celery).—Cut thin slices from a fresh " stalk," *i.e.*, leaf petiole, and place for twenty-four hours in acid Alcohol (1 part of Hydrochloric acid to 2 parts of strong Alcohol). Wash in water and heat gently in a 0·5 per cent. solution of Ammonium oxalate. This dissolves the middle lamella of the cells. Mount a very small portion of the macerated tissue in Glycerine or Glycerine tinted with Erythrosine, cover, and tap on the cover-glass so as to press upon and separate the constituent cells from one another.

The tissue is mainly **parenchyma,** and large numbers of these rounded, thin-walled cells may be seen everywhere. They contain shrunken protoplasts and partly disorganised nuclei. One of the most conspicuous features is the number of **spiral vessels,** some intact and some with the **lignified spirals** unwound. Frequently they are associated in bundles, several of different sizes together, forming **xylem tissue.** A few **netted** or **reticulate vessels** may also be seen, and **pitted vessels** with oval, transverse pits. Vessels are long tubes made of many dead and empty cells joined end to end, and the marks where these cells are joined may be seen as ridges round the vessel at intervals. Where the ends of two vessels adjoin, they may be seen to be pointed and overlapping, so that the line of junction is oblique. Narrow, rectangular **xylem parenchyma** cells may be seen closely adhering to the walls of the vessels.

Close to the xylem strands will be seen **phloem tissue,** with large **sieve tubes,** in which cytoplasm and **sieve plates** may be seen. These cells have thin, cellulose walls, in contrast to the thickened, lignified xylem.

Laburnum anagyroides (Common Laburnum).—Longitudinal slices from a fresh woody stem are soaked in 5 per cent. Chromic acid for twenty-four hours or more, then washed gently in water, after which they can be easily teased out in a drop of Glycerine.

The woody elements shown are very varied, and the object of the maceration is to separate them, so that their individual forms may be better appreciated. The following types can be easily picked out :—

(*a*) TRACHEIDS : both spiral and pitted. Cigar-shaped cells with lignified walls and no cell contents.

(*b*) VESSELS : mostly rather large and pitted, but a few spiral ; also with lignified walls. They tend to break into shorter lengths with square ends, corresponding to the individual cells of which the whole vessel was composed.

(*c*) SCLERENCHYMA : fibre cells, mostly from the xylem. Long, narrow and pointed, with lignified walls and few or no pits. They vary much in length and shape.

(*d*) XYLEM PARENCHYMA : rectangular cells, usually about three times as long as broad, with lignified walls and numerous small, rounded pits. The ends are sometimes drawn out into a little horn on one side, due to overlapping growth of the cells.

(*e*) MEDULLARY RAY CELLS : squarish cells, usually in sheets closely joined together, with thick, finely pitted walls.

(*f*) PITH CELLS : rounded parenchyma, with thin, shrunken walls and little visible contents.

Many other woody stems may be substituted. Old Cabbage stalks are particularly good material.

PART THREE

EVOLUTIONARY MORPHOLOGY

Section 1. Algae

A. CHLOROPHYCEAE

> 1. **Chlamydomonas.**—Material found in a living condition. Mount a drop on a slide and examine first under the low power and observe the active green cells moving rapidly about in the field with a rolling motion. The cells are almost spherical, and it is very difficult to make out the flagella in the living condition.
>
> Take a second drop of the liquid containing the Alga and invert on a slide over a bottle of 1 per cent. Osmic acid for about one minute. Remove and apply a cover-glass. Examine under the low power and observe that the organisms have ceased to move, having been killed and fixed by the Osmic vapour. They are therefore easy to examine under the high power.

The **cell wall** is a thin and delicate layer of colourless **cellulose** bounding the cell. Inside may be distinguished two areas, a colourless upper and a dense, green, lower region. This upper end is sometimes referred to as the **beak.** From the upper end of the cell two flagella pass out through the cell wall (see below), and lying immediately below them are the two **contractile vacuoles,** while to one side a purplish oval body, the **eye-spot,** may sometimes be made out at the upper edge of the chloroplast.

In the lower part of the cell, that is in the posterior region, lies the **chloroplast,** which appears to fill the greater part of the cell. Towards the lower end and in a central position is a spherical dark body, the **pyrenoid.** The chloroplast is usually described as basin-shaped, that is to say it is hollow, and lying inside, surrounded by the chloroplast, is the **nucleus.** For this reason this structure can very rarely be seen. Note that the whole of the remainder of the cell is filled with **cytoplasm,** which is quite colourless.

Place another drop of the liquid containing active cells on a slide and invert over a bottle of 1 per cent. Osmic acid. Leave for a few minutes, to kill and fix the organisms, and then add a drop of a stain made up of 0·5 c.c. of a 1 per cent. aqueous solution of Basic Magenta, 1 c.c. of a 1 per cent. solution of Crystal Violet, and 25 c.c. of water. Leave the stain on and dry in a desiccator for two hours, clean off the stain with Clove oil, wash in Xylol, and mount in Balsam.

In this way the flagella should be stained deep violet, while the organism remains red. The **nucleus** may now be recognized as a violet structure lying more or less centrally in the cell. Cells stained in this way should show clearly the two apical flagella which will be seen to be considerably longer than the cell.

For those who wish for a quick, though less satisfactory method of seeing the flagella, it will be found that by running in a solution of iodine below the cover-glass the flagella will appear as brownish-yellow threads, while the pyrenoid is seen as a dark blue or almost black body lying in an olive-green chloroplast. The nucleus is stained brown or yellow by this method.

Take a fresh drop and fix material showing reproductive stages with Osmic acid, as before. Observe that the contents of the cell have become divided into a number of separate oval bodies which lie grouped in the cell. These are regarded either as **zoospores** or as **gametes,** according to the number present in the parent cell. If the cell has divided into sixteen or less parts each is considered to be a zoospore, and will, on liberation, produce a new individual itself. If, on the other hand, the parent cell has divided into a greater number, thirty-two or sixty-four, each is a gamete and two must fuse together to produce an individual. Each body has a morphology similar to that of the parent, and the chloroplast can be clearly seen. Eventually they will be liberated by the disorganization of the parent cell.

2. **Haematococcus** (*Sphaerella vulgaris*).—Mount a drop of the fresh material on a slide and examine with the low power.

Numerous, active, red or greenish-red cells will be seen swimming actively about in the field. Associated with them are many quite round cells, deeper red in colour, which are without movement.

Kill the organisms in a drop of the material by holding it over a bottle of 1 per cent. Osmic acid and examine under the high power. The active cells will now be seen to possess a very thick gelatinous wall, which is colourless, and inside which is the reddish-green **chloroplast,** which fills the greater part of the cell. Towards the apical end is a small red **pigment spot** or **eye-spot** and two **contractile vacuoles,** close to which will be found the bases of the two **apical flagella,** which pass out through the thick cell wall at an angle with one another and then further diverge. In a living state it may be possible to see that only the terminal parts of these flagella are capable of active motion.

The non-motile cells will be found to have a relatively thin but solid wall and to contain a mixture of green chlorophyll and haematochrome, giving a greenish-red colour, but it is difficult in these cells to make out any organised chloroplast. If the slide is treated with Iodine, black bodies will be found in the cells, indicating the presence of storage **starch.** In active cells a brown **nucleus** can sometimes be made out in Iodine-stained preparations.

It is unlikely that any stages in reproduction will be found in the preparations, but if such do occur it will be seen that in general the process is similar to that already described in *Chlamydomonas*.

3. **Pandorina.**—Mount some of the living material in water and examine under the low power. The colonies of *Pandorina* will be seen swimming about with a rotating motion.

Each colony is dark green and appears to consist of a number of green cells closely arranged, so that the colony looks like a tiny green blackberry fruit (Fig. 5). Examine a single colony under the high power. It will now be possible to make out the separate cells of which the colony is composed. The whole colony is enclosed in a colourless mucilage of smooth outline, through which a pair of flagella emerge opposite each of the component cells. Even without staining it can be seen that the flagella emerge in pairs and that the proximal ends of the flagella are stiff and run parallel to one another. At their anterior ends they diverge sharply from one another, and it is only these ends which are capable of active movement and rotate to propel the organism.

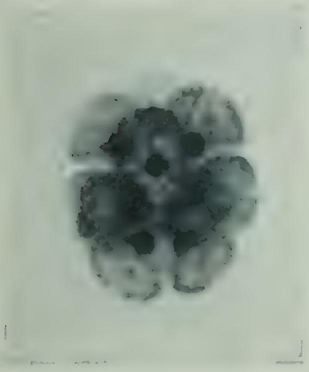

FIG. 5.—*Pandorina morum*, a typical colony.

Each protoplast appears triangular in shape with rounded corners, the apex of each being directed towards the centre. The outer flat end of each protoplast is pale green in colour, while farther in the colour becomes denser, and darker, and it is here difficult to make out any details. About a dozen cells can be recognized in a surface view of the colony, but actually there are sixteen cells altogether.

Fix another drop of the liquid containing the living cells by inverting the slide over a bottle of 1 per cent. Osmic acid, and then add a drop of Iodine solution. Apply a cover-glass and examine. It is now possible to see the gaps in the mucous sheath through which the pairs of flagella emerge. The nucleus of each cell can also be seen as a tiny, more deeply stained, brown body, lying centrally in each cell. It is, however, difficult to gain much information regarding the centre part of the colony owing to its density.

Mount another drop of the material on a slide and look for stages in reproduction. These are not easy to find, but young colonies which have not yet become motile may be found, in which the full complement of cells has not yet been produced. In these the cells are more oval in shape and more widely spaced in the mucilaginous investment, suggesting that the triangular shape assumed by the mature protoplasts is the result of mutual pressure by the adjacent cells of the colony.

4. **Eudorina.**—The colonies of *Eudorina* are larger than those of *Pandorina* and are best mounted in a well-slide. Examine a drop of the water containing the colonies under low power.

It will be seen that they move slowly, by a rotating movement similar to that of *Pandorina*. The cells which compose the colony are round and deep green in colour.

Kill a colony by holding a drop on a slide for a few minutes over a bottle of 1 per cent. Osmic acid and examine under the high power. The cells of the colony are enclosed in an oval mucilaginous envelope which is almost transparent and structureless. The cells of the colony are spherical and large and appear to be arranged in two layers, for a second series of cells can be seen below the first by careful focusing. There are in all thirty-two cells in the colony.

Run in a little Iodine, which will stain the central nucleus yellowish brown in each cell. It is difficult to make out the details of the structure of the chloroplast, which appears to fill the greater part of the cell.

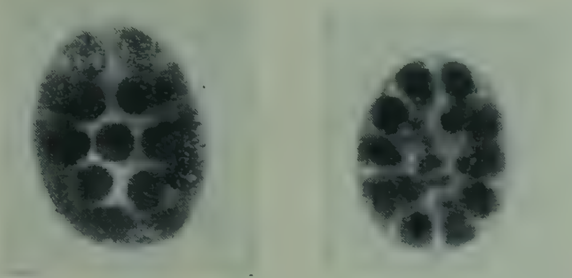

Fig. 6.—*Eudorina elegans*. Surface and sectional views of colony.

It will be seen that in comparison with *Pandorina*, the cells are widely separated from one another and are therefore able to maintain their individual shape (Fig. 6). From the apical end of each cell a pair of flagella pass out through the mucilaginous investment, running more or less parallel and then finally diverging abruptly, the apical ends only of these flagella being actively motile.

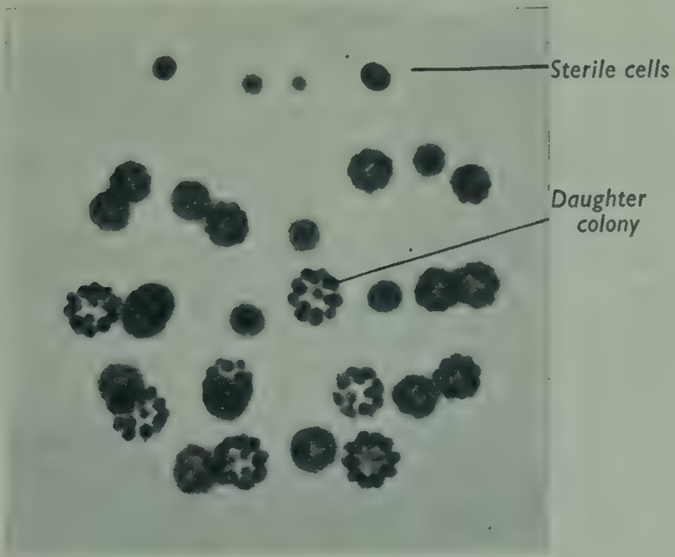

Fig. 7.—*Eudorina illinoisensis*. Formation of autocolonies.

Young colonies are formed within the mucilaginous investment of the parent colony by division of each of the cells into thirty-two (Fig. 7). After liberation they can be recognized by the smaller size of the component cells and mucilaginous investment, but it will be seen that in all these young colonies the number of component cells is the same, no cell increase taking place during growth, since the total number becomes differentiated at the initial stage in the formation of the colony.

5. **Volvox** (*Vegetative Structure*).—Mount some of the living colonies in water in a well-slide, and examine under the low power of the microscope.

Observe that the organism is spherical and is propelled through the water with a rolling movement. It is made up of a very large number of small green cells embedded in the periphery of a sphere of colourless mucilage.

Take another drop of the material and invert over a bottle of 1 per cent. Osmic acid. Examine a single colony under the high power. The colony is now seen to consist of a very large number of unicellular organisms. Each cell is roughly spherical, and is bounded by a very thin membrane. It contains a **chloroplast** which fills the greater part of the cell. Embedded in the chloroplast is a highly refractive body, the **pyrenoid.** Run some Iodine under the cover-glass and note that the pyrenoid is turned black, showing the presence of **starch.** The cells are connected together laterally by a number of **fine processes** which pass through the intervening mucilage. There are usually six of these processes given off by each cell.

Stain and then mount in the same way as advised for the flagella of *Chlamydomonas*, and observe that from each cell there pass out two long **flagella,** each of which is about three to four times as long as the protoplast. The **cell walls** may also now be seen faintly stained violet. They lie in the mucilage between the protoplasts, and the protoplasmic connecting threads pass through them, showing a very slight constriction at this point.

Squash a colony and observe that it is hollow, the layer of cells being quite thin and delicate, with the result that any rough treatment may cause the colony to collapse and become distorted in contour.

Reproduction: Sexual.—If sexual material is available, fix some with Osmic acid and mount on a slide. Observe that certain of the cells are larger than the rest ; these are the **oospheres**. Other larger cells will be seen which possess a thick, slightly spiny wall ; these are **oospores.** Occurring in the same colony, or in different ones, may be found small packets of brown filiform bodies each of which possesses two flagella. These are the **antherozoids,** which are formed from enlarged cells, the **antheridia.** The oospore is formed as the result of the fusion of an oosphere and a single antherozoid.

Reproduction : *Asexual.*—Most collections of *Volvox* show only daughter colonies which appear to be formed asexually. Stages in this process can be easily followed. A single cell of the colony enlarges and becomes darker, due to the accumulation of food material. This is called a **parthenogonidium.** It is divided repeatedly, and careful observation will reveal numerous examples composed of four or more cells lying apparently still attached *inside* to the mucilage of the colony. The number of these daughter colonies in each colony of *Volvox* varies, from four to a dozen generally occurring. They vary in size from those comprising only a few cells to those nearly half as large as the parent colony. They are spherical in shape and hollow, and as they grow the distance apart of the protoplasts increases, due to the enlargement of the cell wall and the deposition of more mucilage. During development the hollow sphere becomes everted, so that its inner surface becomes the exterior of the daughter colony. In general the larger colonies have the larger and therefore older daughter colonies inside them. If a colony is split open by slight pressure on the cover-glass, the daughter colonies will slip out, showing that except at a very early stage they are lying freely in the inside of the parent colony.

6. **Ulothrix.**—Mount some of the filaments in a drop of water and examine under the highest power of the microscope available.

Ulothrix, although an important and interesting type from a theoretical standpoint, is a disappointing species for practical study. The filaments are very fine and composed of very small cells, being as a rule as narrow or narrower than they are long. Each cell possesses a fairly thick **cell wall,** enclosing a single parietal **chloroplast** consisting of a plate curved around the walls of the cell. With careful focusing it should be possible to see this. If the slide is stained with Iodine a single **nucleus** may be identified in the centre of the cell within the curve of the chloroplast. The filaments show a great tendency to break up into small sections of anything from six to a dozen cells in length, especially if the plant is growing under unfavourable conditions.

It is unlikely that any stages of reproduction will be observed, for only under exceptionally favourable conditions, do they appear to be produced. It is not, therefore, proposed to describe these structures in detail here. Should they be observed, the student is referred to a theoretical textbook for a description of them. In any case, it will be only by careful detailed observation, using a high magnification, that it will be possible to distinguish the gametes from the zoospores, for the evidence indicates that the fate of the products of the vegetative cells depends upon the number which are produced from the cell, and hence on their size. If the number is large they fuse in pairs and function as gametes ; if the number produced is small each is capable of independent development.

7. **Pleurococcus.**—Mount in water a little of the fresh material which may be obtained by scraping the green film off a damp wall or tree branch. Examine under the high power.

The green cells of the Alga are grouped together in irregular masses, sometimes only in twos or fours, but more often in greater numbers. If the material has been obtained from a very damp situation the cells may be arranged in short filaments.

Examine an individual cell and note the thin cell wall and the large parietal **chloroplast,** which may appear almost to fill the cell or may be internally lobed. Run some Iodine in under the cover-glass and note that the chloroplast and cell wall are now stained brown. No black spots appear on the chloroplast, thus indicating that no pyrenoid is present.

Reproduction is effected solely by the division of the cells into two parts, and pairs of cells of different sizes can be seen. The colonial form of this Alga is brought about by the failure of these cells to separate from one another.

Here and there among the groups of cells may be seen fine hyphae, suggesting that in nature this Alga forms a kind of loose Lichen association. It may be noted in this connection that an alga generally referred to this genus is a typical component of many Lichens.

8. **Spirogyra.** *Vegetative Structure.*—Mount a filament or two in water and examine first under the low power.

The filament is made up of a series of cylindrical cells joined together in a single row to form an unbranched tube. Each cell is about twice as long as broad, and the ends of the filaments are rounded.

Examine a single cell under the high power. The **cell wall** is thin and is covered externally by a thin layer of **mucilage,** which by its continual sloughing off, prevents any smaller organisms from attaching themselves to the filaments. The cross walls are often bowed and the sides of the filament are slightly constricted at the points where the cross walls are attached.

In some species each cross wall bears an annular fold. At certain times, especially in spring, the pectic material held in this fold swells, thus expanding the fold and breaking the cells apart. This is apparently a method of vegetative propagation.

The threads of *Spirogyra* are usually free-floating, but they are occasionally attached to a substratum by rhizoid-like branches produced from the basal cell.

The **chloroplast** consists of a spirally arranged band which runs diagonally round and round the inside, close to the cell wall. There may be one or more of these spirals, depending upon the species examined. The chloroplast is irregular in outline and has numerous small darker green bodies arranged along its length. These are the **pyrenoids,** which are the centres of starch production. Careful observation will show that the band is T-shaped in section, with fine cytoplasmic threads running from the inturned limb of the T to the nucleus. The whole chloroplast is embedded in the cytoplasmic utricle.

Run in a drop of Iodine solution under the cover-glass. The pyrenoids will turn black, showing the presence of **starch** at these points. The **nucleus** will now be easily visible as a brown body suspended in the centre of the cell by fine protoplasmic strands which pass out to the periphery of the cell. In the nucleus can be seen a more darkly stained body, the **nucleolus.**

Reproduction.—Mount some material, which has been stained in Cotton Blue, in Lactophenol. In good material all stages in the process of reproduction should be found.

The early stage consists in the development of processes which grow out from the sides of the cells towards those of an adjacent filament. The fusion of the cells of adjacent filaments takes place by means of the formation and adhesion of these **conjugation tubes.** This is called the " ladder type " of conjugation. The cell wall of the tube appears similar to that of the vegetative cell wall, the diameter being slightly less than that of the cells of the filament. Only a small aperture is formed in the septum between the cells.

At this stage the chloroplasts of both cells will be seen to lose their clear form, while the cytoplasm contracts to form a more or less globular body in the centre of the cell. This is brought about by the action of contractile vacuoles in the cytoplasm, which pump out water from the central vacuole of the cell. Stages in the migration of the contents of one cell into the conjugating cell of the other filament are infrequently seen, as the movement seems to be pretty rapid. As the " male " protoplasm forces itself through the small connecting aperture it is turned inside out. It then fuses bodily with the cell contents of the " female " cell.

In more mature material all the cells of one of the conjugating filaments will be found to be empty, while the cells of the other contain rounded bodies which at first are green in colour, in fresh material ; or deep blue if stained in Cotton Blue. These are **zygotes.** In material at a still later stage these will be found to have turned into mature **zygospores.** The latter can be recognized readily by the thick yellow wall, which shows very little inclination to stain with Cotton Blue. In material at this stage it will be seen that there is a tendency on the part of the conjugating filaments to become separated, that containing the zygospores being seen with the torn ends of the conjugating tubes still attached. The less common " chain type " of conjugation may sometimes be found, in which adjoining cells of the same filament conjugate by short curved tubes.

Germination of the zygospores may occasionally be found by examination of mud from the bottom of a pond where *Spirogyra* grows readily. If these are found it will be observed that the thick zygospore wall splits partly open, and that the new cell which grows out to start another filament is completely organized with spiral chloroplasts. The young filament grows by division of the terminal cell.

9. **Vaucheria.** *Vegetative Structure.*—The material for studying this alga can usually be obtained by collecting the mats of green threads which may be found on the soil in flower-pots kept in a moist, cold greenhouse. A mat should be washed out carefully in water and a small portion teased out with needles on a microscope slide and mounted in water. Examine under the low power.

The filaments of the Alga are sparsely branched, and in many the cell contents are absent and the cells distorted and collapsed. Select for study a filament which is filled with green matter and is alive and healthy. The cell wall is thin and the ends of the filaments are rounded. Internally the filaments are devoid of any cross walls, that is to say, the construction is **coenocytic.** Each filament contains very numerous, small, disc-shaped chloroplasts, which in surface view appear oval, but, as seen at the sides in profile, they are thin and flat. Run in some Iodine solution, and note that both the cell wall and the chloroplasts are stained brown. Here and there in the coenocytes may be seen more deeply stained, smaller bodies, which are the nuclei.

Mount some more of the material in a few drops of 1 per cent. Osmic acid, cover with a cover-glass and examine under the high power. Scattered through the filaments will be seen numerous bodies which glisten and have stained a greyish black. These bodies are smaller than the chloroplasts and consist of oily material used as a food reserve instead of the more usual starch. Most species of *Vaucheria* are attached to a substratum, and the base of the filament usually sends out short colourless branches which are cut off from the filament by cross walls. These form the **holdfasts.**

> *Reproduction: Sexual.*—Material collected from terrestial situations, or grown on damp mud in a glass dish in bright light, usually exhibits plentiful sexual reproduction. Mount some in water and examine under the low power.

The sex organs are usually borne on short side branches of the coenocytes, or are occasionally sessile. The **antheridium** is a coiled tube, which is developed laterally and is somewhat narrower than the vegetative filament which bears it. The contents of the antheridium become segmented into numerous minute **antherozoids.** The **oogonium** is terminal on the reproductive branch, and is developed only slightly apart from the antheridium. It is a swollen structure, with a prominent beak on the side nearest to the antheridium. The beak opens, forming an entrance for antherozoids. Both sex organs are cut off from the parent filament by walls at the base. The oogonium is densely filled with chloroplasts and oil globules, but immediately within the open beak is a colourless area, the **fertilization spot.**

At a slightly later stage the antheridium may be seen as a coiled tube apparently devoid of contents, while the adjacent oogonium has become even more densely filled. The contents of the oogonium, which previously had been covered only by a thin membrane, are now enclosed by a thick wall, indicating that fertilization has occurred, and that the oogonium has been converted into an **oospore.** If this oospore is examined under the high power it will be noticed that the closely packed chloroplasts, though similar in shape to those of the vegetative filaments, are noticeably larger. It is probable that many free oospores will be observed among the material, if considerable sexual activity is going on, and that filaments with empty antheridia and only the remains of the oogonial stalk will be observed. Such filaments will be more or less devoid of chloroplasts and in process of disorganization.

Asexual Reproduction in *Vaucheria* is by means of **zoospores.** They are not easily produced under laboratory conditions, but it is recommended to cultivate the filaments in 0·1 per cent. Knop solution for a week, transfer to tap water and place in the dark. Zoospores may appear within two days. An alternative method is to place material in a 2 per cent. Sucrose solution in the dark. Bright light and a temperature above 15° C. hinders the formation of asexual reproductive structures.

Should these be found, they will be easily recognized as large swollen **zoosporangia** occurring at the apices of the filaments. When mature the zoosporangium bursts at the tip, and there is liberated a single large **zoospore** provided with numerous paired flagella which radiate from all over the surface. This giant zoospore is provided with a large store of food material and chloroplasts. It is very lethargic and soon settles down, producing a new filament. Theoretically, it is considered that the zoospore represents a number of small biflagellate zoospores which fail to separate from one another.

10. **Oedogonium.** *Vegetative Structure.*—Mount some of the fresh material in water and examine under the low power. The alga consists of unbranched filaments composed of a single row of cells. The filaments are fine and appear uniform in outline except where reproductive organs occur. Examine the preparation under the high power.

Each cell of the filament is about three times as long as wide. The cell wall is thin, but at one end of many of the cells may be seen several **apical caps** appearing as a succession of closely associated transverse ridges, while the side walls of the cell in this region appears to be successively notched. These are the dividing cells, which grow by the opening out of a ring formed inside the wall, near one end, followed by the formation of a new cross wall. The ridges mark the position of successive rings.

The cells each contain a reticulate, parietal chloroplast which presents the appearance of many small, disc-shaped, dark green chloroplasts with delicate connections, lying around the periphery of the cell. One end of each cell is slightly larger than the other, so that the outline of the filament is not quite uniform. Now fix the material by adding a drop of Iodine solution and examine again. The dark colour of the chloroplast indicates the presence of starch, and here and there may be seen small black bodies, the **pyrenoids,** lying in the chloroplast. A relatively large single nucleus may be seen lying near the centre of the cell. This may be made out especially clearly in those cells in which the chloroplast has become disorganized. This nucleus is spherical in shape and appears to be suspended in the cell by extremely fine protoplasmic threads.

Reproduction: Asexual.—This occurs chiefly in the autumn, and suitable material may be obtained at this time.

Certain cells of the vegetative filaments will be seen in which the contents are more dense and form an elliptical body lying in the cell. At a slightly later stage the filament will be seen to have broken partly across, generally at the lower end of a series of cell caps, and from the opening emerges the single large **zoospore.** The whole process can be followed under the microscope if suitable material is obtained. When material is brought indoors the warmth of the laboratory may start the process. The zoospore appears for a time to lie enclosed in a very thin membrane, but finally it escapes as a spherical body, densely filled with pigment and food material, except for a clear apical end. Surrounding this end is a complete ring of very short flagella, by means of which the zoospore swims sluggishly about in the water.

Reproduction : *Sexual.*—Mount some material with dwarf males, which has been stained in Cotton Blue, in Lactophenol.

The **oogonia** are large, more or less spherical cells, developed at intervals along the filaments, and containing a single **oosphere.** Each oogonial cell is larger than the adjacent vegetative cells and has a thin cell wall with a particularly thin area near the upper end, which opens over the **receptive spot.** Very frequently the oogonium develops from a cell with a series of apical caps, and these can often be found forming a collar above the oogonium.

The dwarf males, or **nannandria,** are found growing either directly on the oogonium or close to it (Fig. 8). Each consists of two or three cells, the lowest of which is elongated and has a rhizoidal outgrowth by means of which it is attached to the filament. Above this are usually two flat cells, the **antheridia,** from each of which two **antherozoids** are produced and liberated by a lid-like opening. The dwarf male filaments arise from **androspores** formed in **androsporangia,** which may originate either in the same filaments as the oogonia or in different ones.

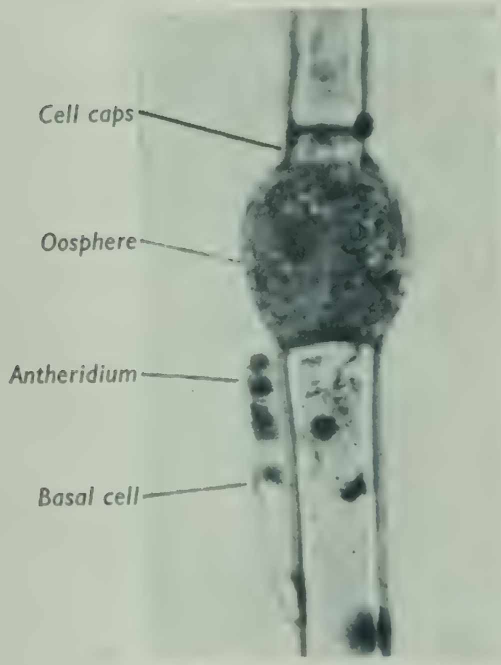

FIG. 8. *Oedogonium crassiusculum.* Oogonium with nannandrous male plant.

Later stages will show the result of fertilization. The oosphere has become converted into an **oospore,** with a thick, almost colourless wall and reddish contents, due to the abundance of oil, formed presumably as a food reserve.

Germination of the oospore is apparently facilitated by frost. The oospore loses its red colour and divides into four flagellated bodies which escape through an opening in the wall.

In some species, instead of the formation of dwarf male filaments, the antheridia are produced directly from vegetative cells on either the same or on different filaments from the oogonia, and no androspores are formed.

B. CYANOPHYCEAE

Nostoc. *Vegetative Structure.*—There are a number of common species in Britain, some of which are terrestrial and others are found in water in mucilaginous masses. Each of these masses is more or less globular and slimy to the touch.

Squash out a portion in a drop of water, stain lightly in Cotton Blue and mount in Lactophenol.

The mucilage contains a vast number of short filaments of cells which are concentrated mainly around the periphery of the mass. Each filament, which is referred to as a **trichome**, consists of a chain of about a dozen to two dozen rounded cells, surrounded by a distinct gelatinous sheath. These trichomes are twisted or contorted in various ways and are made up of cells only loosely connected together. Each trichome possesses one special cell termed the **heterocyst**, which can be recognized by its considerably larger size, thicker wall, and colourless contents. In the longer filaments the heterocyst is intercalary, situated about the middle of the filament. Short filaments will also be seen, some with a terminal heterocyst, some with none. These short filaments have been produced by the division of the long filaments, which undergo **hormogone formation.** This consists in the splitting of the original filament at the point between the heterocyst and the next vegetative cell. The result will be two filaments, one with a heterocyst, the other without. By growth the missing part will later develop. The large number of filaments in the colony is due mainly to hormogone formation. The vegetative cells of the trichomes contain **metachromatic granules** which are seen as bright shining bodies in the centre, surrounded by the chloroplasts.

In some trichomes certain cells will be seen to be larger than the remainder. These cells may have divided each into four **akinetes.** When these are liberated each will produce a new trichome.

C. RHODOPHYCEAE

1. **Batrachospermum.** *Vegetative Structure.*—Fix a small quantity of the material in Chrom-acetic solution, wash thoroughly in water and then stain in Cotton Blue in Lactophenol. Mount in Lactophenol.

The algal thalli are made up partly of longitudinally running filaments and partly of branching lateral filaments, which are produced in clusters at intervals along the longitudinal filaments. The cells of the longitudinal filaments are very long and narrow, about a dozen filaments being grouped together to form the main axis. The lateral branches are composed of much shorter cells, which are transversely wider at the distal than at the proximal ends. They are jointed, and connected together by narrow, fine, protoplasmic connections. The cell contents are finely granular, but it is difficult to make out internal detail. Among these lateral branches may be seen large clusters of **carpospores,** arising as the result of fertilization. The carpospores are smaller than the cells of the branches and are spherical, with dense contents and a thin cell wall. Each has a single spherical nucleus lying in the centre.

At the apex of the axis the lateral filaments are densely clustered together, showing that the subsequent separation of the lateral branches is due to secondary elongation of the cells of the axial filaments. Branching of the main axes rarely occurs, but in the lower part of the thread the central filaments become somewhat spirally twisted, and many of these cells lose their contents. In this region it can be seen that the main axis consists of an enlarged central filament composed of cells devoid of contents, around which there are twisted a number of much finer filaments which are pigmented. The lateral branches arise from these peripheral filaments. Branching of the main axis arises from a division of the cells of the central filament.

Reproduction: *Sexual.*—The reproductive organs in *Batrachospermum* consist of an antheridium (male) and a procarp branch (female).

In average material it is probable that the stage most commonly occurring will be a post-fertilization stage which results in the production of the carpospores. These can be seen in most preparations as a more or less spherical group of rounded cells attached to the finer branches of the thallus. These carpospores are cut off from the ends of short **gonimoblast filaments** formed from the fertilized carpogonia.

In well-stained material it may be possible to find, near the apex of the filaments, the **carpogonium,** which may be recognized by the long thin **trichogyne** attached to its distal end. It will be seen that each carpogonium terminates a short branch of two or three cells which is the **procarp branch.**

The **antheridia,** producing the **spermatia,** are also produced on short branches of the lateral filaments. They may be recognized by the small clusters of minute spermatia budded-off from their apices.

2. **Chondrus crispus**. *External Morphology.*—There is only one British species but it varies considerably in size and shape. Some specimens are only about three inches long and almost circular in section, others may be six or more inches long and the upper segments considerably flattened in section. The colour may vary from purple-red to red, depending upon the depth of water in which the plant is growing. It is common on rocky coasts around Britain, especially in the north and west.

Examine a plant in water in a dish.

At the base is an **attaching organ,** a flat plate of tissue which adheres closely to the substratum and may have some fragments still attached to it. It is almost black in colour and bears the flattened shoots. Each shoot is at first undivided, but after a short distance dichotomizes into two equal halves. This may be repeated several times, so that a fan-shaped thallus is built up. The reproductive organs appear as somewhat swollen patches near the tips of the branches. The sexes are separate and so are the tetraspore plants, though they are all morphologically alike. The sexual plants are fertile from October to December. The asexual plants produce their reproductive organs between December and March.

Structure of the Vegetative Thallus.—Cut a transverse section through the thallus, stain in Cotton Blue and mount in Lactophenol.

In the centre will be found long, narrow **central cells,** which have fairly thick walls and form an interwoven tissue embedded in mucilage. Passing out from the centre are the **collecting cells** which are considerably shorter and form a peripheral zone around the central cells. Around the margin of the section will be seen the **assimilating cells,** which lie parallel to one another and terminate at the surface of the thallus in somewhat swollen ends. They are closely set side by side. This superficial zone is rich in chloroplasts and in phycoerythrin pigment.

Reproduction : The Nemathecium.—Cut a transverse section through a frond so as to pass through a nemathecium. Stain in Cotton Blue and mount in Glycerine.

The **nemathecia** appear as swollen areas on the flattened fronds, each being surrounded by the outer tissue of the thallus. Inside the swelling will be found large numbers of somewhat globular cells which are rich in protoplasm.

Depending upon whether the plant is a female or a tetrasporic one, the structure of these central bodies will vary. The **carpospores** are roundish bodies containing much cytoplasm and a large amount of starch. In fresh material they are deep red in colour. The **tetrasporangia** are at first developed in rows but this system is soon lost and the tetrasporangia come to lie freely in the cavity of the nemathecium. At a later stage each tetrasporangium will be found divided cruciately into four **tetraspores,** which finally separate and become spherical.

The antheridia are not produced in nemathecia but on segments of the thallus among the assimilatory cells. Each consists of a short filament of almost colourless cells from each of which one **spermatium** is produced.

3. **Polysiphonia.** *Vegetative Structure.*—Mount some apical portions of the fresh material in sea water.

The thallus is richly branched dichotomously, each branch terminating in an apex possessing a single, large, lens-shaped, terminal cell, followed by a number of flat cells containing chloroplasts but very little red pigment. In the apical cells the nuclei can be clearly seen.

In the mature thallus the branches are made up of a series of parallel filaments, or " siphons." The **central siphon** is composed of large barrel-shaped cells, and it is surrounded by a number of **pericentral siphons** composed of thinner, elongated cells. Each cell of the central siphon is surrounded by a cortex layer of a definite number of pericentral siphons all terminating at the same level, and thus forming a series of segments in the axis. Each cell contains a single nucleus and many small discoid **chromatophores.** The red pigment is contained in the chromatophores, but diffuses throughout the cell at death. Examination of the growing apex will show that the cells of the centre siphon are cut off by transverse divisions, parallel to the lower wall of the apical cell, the separation of the pericentral siphons not occurring till some time later by a series of successive longitudinal divisions. The apical cell divides into two by a longitudinal division prior to the dichotomy of the branch.

> *Sexual Reproduction.*—Stain some material of *Polysiphonia*, bearing antheridia, in Cotton Blue and mount in Lactophenol.

The **antheridia** are borne near the apices of the branches. Each consists of a central filament of cells which arises from the central siphon and gives off very short lateral branches of cells all round. It is from the terminal cells of these that the spermatia arise. The whole antheridium is oval in form, and slightly curved. It is entirely naked, and the spermatia as they are budded off, escape directly into the water. Each **spermatium** is a tiny spherical body devoid of a wall and of flagella, and therefore of the power of motility. It relies on the water currents to bring it into contact with the trichogyne of the female organ.

> Stain some material of *Polysiphonia* bearing cystocarps in Cotton Blue and mount in Lactophenol.

The **cystocarp** is an oval or urn-shaped body, attached to the side of the filament and having an opening at the upper end (Fig. 9). The margin of the opening may sometimes appear turned back and frilled. The wall of the cystocarp is composed of a single layer of cells. Unless sections of the cystocarp can be cut it is unlikely that any stage in the development of the procarp branch inside it will be made out and, in any case, the earliest stages are produced before the formation of the cystocarp. These must be looked for close to the stem apex at the level at which cortex formation is going on. The only structures which can be recognized clearly in the cystocarp are the fully developed **carpospores.** By careful focusing these may be seen through the wall of the cystocarp. Each is an oval body, and they are arranged in a single spherical layer, at first attached by their inner ends but later becoming free. They are liberated through the mouth of the cystocarp.

> *Asexual Reproduction.*—Stain some material of *Polysiphonia* bearing tetrasporangia with Cotton Blue and mount in Lactophenol.

The **tetrasporangia** are borne on different plants from the male and female organs, though in general appearance the two generations are similar. The tetrasporangia arise from the pericentral cells near the apex of a filament and are generally formed in longitudinal series. Each tetrasporangium is supported on a stalk and consists of a single spherical tetraspore mother cell. When mature the nucleus divides by reduction division (meiosis) into four, and cross walls are laid down dividing the mother cell into four **tetraspores.** When mature they are liberated by the breakdown of the sporangium wall.

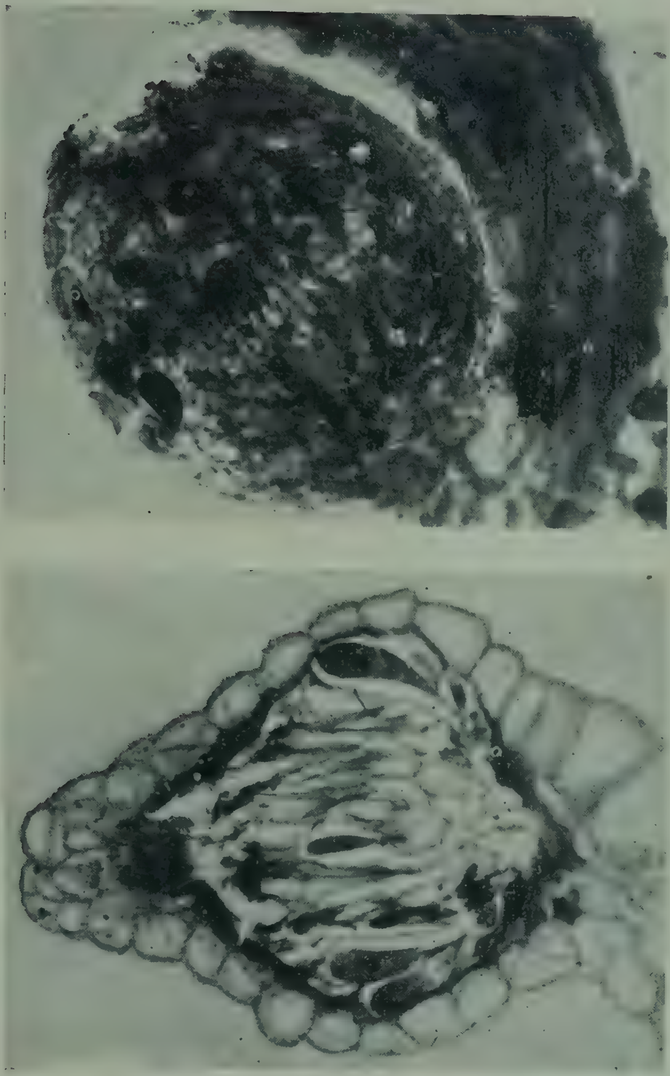

Fig. 9.—*Polysiphonia nigrescens.* Cystocarp in surface and sectional views.

4. **Corallina officinalis.** *External Morphology.*—This is the commonest British species, which is found chiefly in rock pools around the coast. The colour of the thallus varies with the depth of water and to some extent upon its mineral covering, but is usually pink. Generally the thallus is thickly encrusted with lime, which must be removed by treatment with dilute Hydrochloric acid before the structure can be successfully studied.

Examine a decalcified plant which has been washed free of acid and is lying in water. The removal of the lime makes the thallus very delicate and it must be handled with great care.

The thallus is made up of a number of articulated segments which are monopodially branched, two branches normally arising from the upper end of each articulation of the main axis. The articulations are usually broader at the top than at the bottom, the upper surface of each articulation therefore allowing for the attachment of the joint above as well as for the two lateral branches. The whole thallus has a tufted growth and reaches a height of not more than six inches; frequently it is considerably less. It arises from a calcareous disc which is attached to the rock on which it grows, and numerous thalli may arise close together to produce a large tuft. It grows both on rocks and epiphytically on other seaweeds.

The various reproductive organs are borne on separate plants but always in conceptacles. These conceptacles are formed terminally in the uppermost segments of the main branches. They enclose **antheridia, carpogonia** and **tetrasporangia** respectively.

> *Microscopic Structure of the Thallus.*—Mount a few decalcified joints of the thallus in Lactophenol, after staining in Cotton Blue.

In the centre the thallus consists of parallel rows of narrow elongated cells forming a fairly solid tissue. These filaments of cells fan out towards the margin where they terminate in slightly swollen cells. The central cells are thin walled and are only sparsely filled with cytoplasm. The marginal cells have thicker walls which stain more readily and the terminal cells are filled with plastids and form a uniform peripheral zone.

The construction of the **joint** between two segments is different from that of the segments themselves. The cells of the joint run parallel longitudinally, and they have very thick walls which stain readily. Each cell is very narrow with pointed ends and forms a tissue reminiscent of a group of fibres. Collectively it forms an almost cubical block of tissue. The cells of the joint are in communication with the central cells of the segments above both and below, though one filament of the lower articulation may divide to give two cells of the joint. These joint cells are closely packed together, whereas the central filaments of the segments are not, spaces filled with mucilage occurring between them.

> *Reproduction: Longitudinal Section of a Male Conceptacle.*—Using decalcified material, cut a median longitudinal section through a male conceptacle. Stain in Cotton Blue and mount in Lactophenol.

The section will show the **conceptacle** formed in the upper part of the segment and replacing the central filaments. Externally it is lined by filaments of the segment, which grow inwards above and form a neck to the conceptacle which is open at the top. The **antheridia** arise in groups of two or three from **antheridial mother cells,** which form a layer in the base of the conceptacle. These antheridial cells are very long. Each consists of a terminal portion containing the nucleus attached to a very long tail. They become detached and some may be found lying loose inside the conceptacle before they have been liberated. They appear to function as spermatia.

> *Reproduction. Longitudinal Section of the Female Conceptacle.*—Using decalcified material, cut a median longitudinal section through a female conceptacle. Stain in Cotton Blue and mount in Lactophenol.

The female conceptacle is formed in the same position as the male and the conceptacle is built up in the same way but it is often larger and may occupy the whole of the centre of the segment; moreover the terminal segments of the adjacent lateral branches may also form conceptacles.

Inside the conceptacle it is unlikely that stages in the development of the procarp branches can be made out and only in mature conceptacles will it be possible to study the contents. In such conceptacles the lower part will be filled with the large oval **carpospores** each of which is filled with dense cytoplasm and contains a rather large, conspicuous nucleus. When mature the carpospores are liberated through the mouth of the conceptacle.

Reproduction. Longitudinal Section through a Tetrasporic Conceptacle.—Using decalcified material, cut a median longitudinal section through an asexual conceptacle. Stain in Cotton Blue and mount in Lactophenol.

Tetrasporic conceptacles are formed in the same manner and in the same position as the sexual ones. When mature there is formed in the base of the conceptacle a group of large cells, each with dense cytoplasm and a conspicuous nucleus. These are the **tetrasporangia.** At a later stage these tetrasporangia will have become elongated and have each divided by cross walls to form a row of four **tetraspores.** This method of division of the mother cell to form four spores arranged in a single row is termed **zonate.** It is not very common among Algae but is characteristic of *Corallina.*

The tetraspores are liberated singly through the apex of the conceptacle.

D. PHAEOPHYCEAE

1. **Ectocarpus.**—There are many species of *Ectocarpus.* The one selected for description is *E. confervoides*, a species common around the British coast. Mount in Lactophenol and examine some of the material which has been stained in Cotton Blue.

The filaments are branched. Each lateral branch tapers to a point which is not prolonged into a hair or trichome. The cells are slightly wider than long, and the thin cell wall is constricted at the point where the cross walls occur. Each cell contains a number of more or less globular **chromatophores** arranged around the periphery of the cell, each with a small shining body in the centre. Suspended in the centre of each cell is the nucleus. Here and there may be found much shortened cells resulting from repeated cell divisions, indicating that any cell of the filament is capable of division and that growth is effected, not by an apical cell, but is **intercalary.**

Reproduction.—Attached to the filaments, particularly on the lateral branches, may be seen cells which are considerably larger than the rest. These cells have more dense contents and stain more readily with Cotton Blue. They are **unilocular sporangia.** Frequently these sporangia are produced in groups of ten to twenty, some showing protoplasmic segmentation. Each sporangium gives rise to a number of kidney-shaped **zoospores**, which finally escape through a lateral opening.

On separate plants the plurilocular, sexual **gametangia** may also be found. These are elongated structures, slightly wider in diameter than the cells of the filament, and are attached laterally to the main filaments. Each is made up of a large number of very small, rather rectangular cells, each of which produces a single motile, kidney-shaped gamete. The gametes, generally from different gametangia, usually fuse in pairs to form a zygote.

> 2. **Cutleria multifida.** *External Morphology.*—The plant occurs only on the south-west coast of England, where it is found attached to rocks in deep water.

The thallus is richly but irregularly branched, and grows from a small, flat holdfast. The lower branches are small and rounded, while those above are flat and somewhat broadened before branching occurs. This branching is sometimes more or less dichotomous but is really brought about by a splitting of the thallus, so that the segments produced may be unequal in width. The tips of the branches terminate in very fine points, the construction of which can be seen only under a microscope and will be described below.

The surface of the thallus is covered with groups of fine hairs, among which the reproductive bodies occur. They are produced all over the thallus except near the apices.

> *Structure of the Thallus.*—Mount a small portion of the thallus, including the apex of a shoot, on a slide, stain in Cotton Blue and remount in Lactophenol.

The apex of the shoot is peculiar and is not comparable with that of other types of Phaeophyceae. Each apex terminates in a cluster of long filaments made up of a single row of cubical cells, each containing a large nucleus and numerous small plastids. At or near the base of each filament is a zone in which the cells are much narrower and it is here that active cell division is taking place. Such a method of increase in length by the division of intercalary cells of a filament is called **trichothallic growth.**

Further back, below the growth zone, the filaments coalesce together to form a solid tissue made up of cubical cells similar to those of the filaments, which are all bound together by mucilage. At first these cells show the same uniform arrangement which was seen in the filaments but farther back they lose their regularity.

In a section of the thallus farther back it will be seen to consist of a central tissue of large polygonal cells with thin walls, surrounded by several layers of smaller, more rounded cells with slightly thicker walls. The surface is made up of a single layer of very small, thick-walled cells, in which are contained the plastids, and it is mainly this layer which functions as the assimilatory tissue for the plant.

Sexual Reproduction : Male Gametangium.—Cut a transverse section through the male plant in the region of the gametangia. Stain in Cotton Blue and mount in Lactophenol.

The male gametangia are produced in large clusters, which are scattered on the surface of the thallus of the male plant, being chiefly developed towards the upper end of the main branches. Each **sorus** is made up of a large number of branched filaments some of which give rise to **paraphyses.** The others bear the male **gametangia** which are formed in groups, laterally on the filaments. The cells of the filaments are small and cubical but those bearing the gametangia are longitudinally enlarged. Each gametangium consists of a number of cells arranged in four ranks, there being between 6 and 25 cells in each rank or up to nearly 100 cells in all, which become the male gametes on liberation. The paraphyses are made up of small cubical cells and are of considerable length, enclosing and enveloping the gametangia, and probably serving to protect them during their development.

Though the sori arise from the superficial cells of the thallus they cause no anatomical change in the structure of the underlying cells. The cells of the paraphyses and the reproductive branches contain dense cytoplasm but can easily be distinguished from those of the gametangia, which take up stain much more readily. In the latter are shiny granules and in some a nucleus can be observed.

Sexual Reproduction : Female Gametangium.—Cut a transverse section through a female thallus in the region of the gametangia. Stain in Cotton Blue and mount in Lactophenol.

The female gametangia are formed in clusters on the surface of the thallus. Each **sorus** consists of a number of **gametangia** mixed with **paraphyses.** The female gametangia are stalked, the stalk consisting of about three cubical cells, each with dense contents. The gametangium is rectangular in outline and appears to be made up of about eight cells in two rows. Actually, of course, there are more than this, usually sixteen, arranged in four rows of four cells. The cells become the gametes. Each female gamete is roughly spherical and the cells are dense and stain deeply ; a nucleus may be seen in some of them. The paraphyses are few in number and arise separately, from the superficial layer of the thallus or occasionally from the stalks of the gametangia. They are made up of single filaments of cells, each of which resembles a stalk cell. The terminal cell is rounded and is not drawn out into a trichome.

Asexual Reproduction.—Transverse section of the *Aglaozonia* stage to show the zoosporangia.

The asexual plant of *Cutleria* differs completely from the sexual one. These asexual plants are small and prostrate and the zoosporangia is produced upon the upper surface.

In a transverse section of the thallus it is possible to distinguish a central tissue of large rectangular cells, usually two cells thick, which is bounded above and below by smaller cells. On the lower surface there is a single layer of brick-shaped cells, but on the upper surface there may be two or three layers. Each **zoosporangium** is supported on one, or sometimes two, basal cells, which are slightly broader than the base of the zoosporangium. The zoosporangium is an oval sac with a fairly thick wall. It stains deeply and contains about a dozen laterally biflagellate **zoospores,** which, when mature, escape through the top. These zoospores are nearly spherical with a slight protuberance at one end, and contain several plastids.

The zoosporangia are produced in large numbers close together on the surface of the thallus, thus forming a superficial layer. No paraphyses are produced in connection with the asexual reproductive organs as they are in the sexual ones, but the margins of the sori may be surrounded by one or two rows of sterile cells, smaller than the zoosporangia but produced in the same way. Zoosporangia lying side by side usually liberate their zoospores at the same time.

3. **Dictyota.** *Vegetative Structure.*—Mount in Lactophenol a small apical portion of the thallus which has been stained in Cotton Blue, and examine under the low power.

Dictyota dichotoma is a common Alga along the British coasts. It is greenish yellow in colour and is composed of a thin, narrow thallus several inches long, which is branched dichotomously. At the basal end of the thallus is a small disc-shaped attaching organ. At the apex will be seen a depression between two pointed upgrowths of the thallus. The apices of these points have each a single large **apical cell,** which is the growing point of the branch. Under the high power it will be seen that cells are cut off from the lower and from two lateral sides of this large cell, which, unlike most apical cells, appears to have less stainable material in it than the cells immediately below. These cells are formed by radial divisions, and in consequence lie in concentric circles radiating from the apical cell.

Since the thallus is thin, much of the cell structure can be made out from the surface view, but if a section through the thallus be cut it will be seen to consist of a central layer of large cells, bounded by thin cell walls and having a peripheral layer of small discoid chromatophores and a central nucleus. These cells are all arranged in transverse rows, and their cross walls in surface view form continuous bands across the thallus. On each side of these cells lies a very small-celled tissue, of one layer of cells, also containing chromatophores and nuclei, though the latter are difficult to make out. These cells form the surface of the thallus.

Asexual Reproduction.—Mount in Lactophenol a small portion of the thallus from an older frond showing asexual reproduction, which has been stained in Cotton Blue. Alternatively, cut a transverse section through the thallus in the region of the sporangia.

Examine under the low power and note the round **tetrasporangia,** which are grouped together in irregular clusters all over the surface of the thallus.

Under the high power the structure of the tetrasporangium may be seen. It is enclosed in a thick mucilaginous wall, which in the young state encloses dense, deeply staining contents in which a single darkly staining nucleus can be made out. More mature tetrasporangia will show the division of this nucleus into four, and the cruciate cleavage of the protoplasm into four portions. In the mature sporangium are four **tetraspores,** only three of which will be apparent, though the fourth may be made out by focusing.

Sexual Reproduction.—Cut a transverse section across a portion of the male thallus bearing antheridia, stain in Cotton Blue and mount in Lactophenol.

The **antheridia** are grouped together in **sori.** These sori may be produced on either surface of the thallus. In a section the thallus itself is seen to consist of a central layer of large cells covered on either side by a layer of very small cubical cells, and it is from these small cells that the antheridia arise.

Each sorus is surrounded by two or three rows of elongated sterile cells, and each antheridium possesses a basal cell upon which the elongated antheridium rests. The antheridia themselves are elongated sacs closely packed together, so that by mutual pressure as they enlarge they come to fill completely the space inside the ring of sterile cells. The sterile cells which form the wall of the sorus vary in height, those nearest the exterior being the shortest, those nearest the antheridia the longest. In this way they are best suited to withstand the internal pressure exerted by the expansion of the growing antheridia. In mature antheridia the individual **antherozoids** may be recognized as small, rather spindly structures with two lateral flagella.

Cut a transverse section through the thallus of a female plant and mount as before.

The **oogonia** are produced in **sori** similar to those of the antheridia, but without any sterile cells. Only one **oosphere** is formed in each oogonium, though a number of oogonia are formed in each sorus. The oospheres are liberated into the water as passive bodies, and fertilization by the antherozoids takes place there. If some ripe gametes are introduced into a drop of sea-water it may be possible to see the active antherozoids attaching themselves to the oosphere and whirling it about by their activity.

4. ***Laminaria.*** *External Morphology.*—The external form of the fronds varies greatly according to the species. Three species occur commonly below low tide mark all round the British coast.

In *Laminaria saccharina* the thallus consists of a simple, unbranched strap, divisible into a basal portion which is cylindrical and a long, terminal portion which is flat. The margin may be smooth but is more often frilly and the whole plant is of a uniform brown colour. If, however, it is dead and has been cast up on the beach, it may have partly lost its brown colour and become paler with traces of green. It is attached to the rock by means of a **holdfast,** which consists of a much-branched mass of cylindrical, root-like organs, the tips of which adhere very strongly to the rock surface. The frond grows to 6 ft. in length.

In *L. digitata* the fronds are also divisible into two parts, a cylindrical basal portion arising from the holdfast, and a flat terminal portion which instead of being simple is palmate and is divided into a variable number of separate, narrow segments with smooth margins. It is the largest British species and may reach 15 ft. in length.

In *L. cloustoni* the thallus is somewhat similar to that of *L. digitata* but differs in that the flat portion of the thallus instead of passing gradually into the top of the stipe is almost cordate. The length of the cylindrical portion is generally greater than in *L. digitata.* It is somewhat smaller than the last species and rarely grows to more than 12 ft.

The thalli are all usually perennial and new segments are formed at the base of the flat fronds which remains meristematic and produces a new frond annually. There is generally a constriction between old and new fronds, but eventually the older frond is shed. Epiphytic algae occur on the stipe and it is common to find the bases of old plants smothered in a mass of other Brown and Red seaweeds.

> *Transverse Section of the Stipe.*—Cut a transverse section of the stipe, stain in Cotton Blue and mount in Lactophenol.

The tissue is divisible into two zones, an external **cortex** and a central **medulla**. If the section is taken through a large stipe it may show successive zones of growth, which will be seen only in the cortex. On the outside lies a zone of small cells containing considerable quantities of pigments and forming the **assimilating zone.** Immediately inside this is sometimes found a zone with intercellular cavities which are provided for storage of mucilage. The true cortex is made up of a uniform tissue consisting of thin-walled cells which are radially elongated. The width of these cells in a radial direction is remarkably uniform and could be compared with the radial rows of cells of a secondary wood. The tangential width of the cells, however, varies considerably and there may be present alternating bands of wide and narrow cells, the latter having rather thicker walls than the former.

In the centre the medulla consists of cells more loosely arranged, with spaces containing mucilage between them. The size of these cells varies somewhat. Many are small with thick walls, while in others the lumen is traversed by a plate, perforated with small pits. These are the swollen ends of the **trumpet hyphae.** Here and there these trumpet hyphae may be found cut longitudinally, for the filaments of the medulla are irregular and do not all run in a longitudinal direction.

> *Longitudinal Section of the Stipe.*—Cut a median longitudinal section through the stipe, stain in Cotton Blue and mount in Lactophenol.

The section will show the same division into **cortex** and **medulla** which has been observed in the transverse section, but it is possible to see the tissue differentiation more clearly. The peripheral cells of the **assimilatory zone** are small and rectangular in section with their long axes pointing outwards. Internal to them the cells are of the same shape but become progressively larger towards the inside.

This zone is followed by one in which the cells are elongated in the longitudinal direction, being three to four times as long as broad. The cells have fairly thin walls and contain cytoplasm and nuclei. There is clear evidence of sliding growth as the ends of these cells push between one another as the zone increases in diameter. It is possible to see that the size of these cells varies in successive zones. Those cells which in the transverse section appeared to be radially elongated are the longest, while the small-celled zone is that in which the cells show least sliding growth and are individually shorter. It is these latter zones which, by the growth of the component cells, increase most rapidly the diameter of the stipe.

The cells of the **medulla** are much less regular. They are composed of longitudinally running, branched hyphae and differ among themselves both in width and length. The ends of some hyphae nearest the middle are expanded and join those of neighbouring hyphae, forming long tubes with expanded ends. These are the **trumpet hyphae** and their end walls are perforated by numerous small pits. All these medullary hyphae are embedded in dense mucilage which stains readily.

> *Asexual Reproduction.*—Cut a transverse section through a reproductive frond. These can be recognized by their slightly greater thickness and by their greyish colour. Stain in Cotton Blue and mount in Lactophenol.

A transverse section through a portion of the frond will show its general structure, which may be compared with that of the stipe. In the centre is a narrow zone of **medullary hyphae** which is bounded on both sides by the **cortex.** The cells comprising it are roughly hexagonal and decrease very greatly in size from the centre towards the margin. At the margin is a single layer of very small, almost spherical cells, from which the asexual reproductive organs arise.

These bodies consist of **zoosporangia** which are grouped together in large patches or sori, which may cover considerable areas of the frond. The construction of the reproductive organs is elaborate and can only be made out in really thin sections. From the superficial cells of the thallus, long straight **paraphyses** are formed which may be twenty times as long as the basal cells which support them. The zoosporangia arise from the basal cell which supports the paraphyses, two being produced from each. These zoosporangia are elongated sacs and when mature lie tightly appressed on either side of the paraphysis. The tips of all the paraphyses are expanded and become more or less united together to make a covering over the developing zoosporangia.

Each zoosporangium contains a large number of **zoospores,** which can be seen as deeply stained bodies. It is possible, if fresh material is employed and examined in sea water, that some of these zoospores may be liberated. In this case it will be seen that the zoospores are almost pear-shaped bodies, bearing two long, laterally placed flagella.

5. **Fucus.** *Vegetative Structure.*—There are three common species of *Fucus*, which differ considerably in their external morphology but not in their internal anatomy. *Fucus vesiculosus* is the Common Bladderwrack, and *Fucus serratus* the Serrated Wrack. A third species, *Fucus spiralis*, resembles *F. vesiculosus* but has no bladders.

Examine a mature specimen of *F. vesiculosus*; the plant consists of a flat thallus, dichotomously branched, with a clearly marked midrib. The lower parts are more or less rounded and consist of little more than the central midrib; the younger parts have marginal wings and a less noticeable midrib. Scattered over the flat part of the thallus are numerous inflated bladders containing air. The margin of the thallus is entire and smooth, and the surface of the swollen upper extremities is covered with small pits, the openings of the **conceptacles.** The basal part of the thallus terminates in a rounded disc-shaped **holdfast.**

In *Fucus serratus* there are certain marked differences. There are no bladders on the thallus, the midrib is less clearly marked and the margin of the thallus is serrated. The apical ends of the thallus in the region of the conceptacles are only slightly thickened, but in other respects the two resemble one another closely.

Cut a transverse section through the flat part of the thallus, stain in Cotton Blue and mount in Lactophenol.

The outermost layer consists of small, rectangular cells, fitting closely together and filled with plastids of a dark brown colour. This colour is due to **fucoxanthin,** a carotinoid pigment which conceals the presence of chlorophyll. If a portion of the thallus is immersed in fresh water the brown pigment dissolves leaving the chlorophyll visible.

This surface layer is the chief seat of photosynthesis. Within it there lies a zone of rounded cells, the size of which increases towards the centre of the section. These contain fewer plastids and form the **cortex.**

The internal tissue is formed by narrow, filamentous cells separated widely from each other by intercellular mucilage. This is the **medulla.** The filaments are thin walled, but among them are intertwined many thick-walled filaments, which originate from the cortex and form a mechanical support for the thallus.

Cut a longitudinal section through the upper part of the thallus, passing through the midrib region. Stain, and mount in Lactophenol.

The cell filaments in the medulla will be seen to run more or less straight longitudinally, but the thickened filaments which come from the cortex follow a very irregular course. Their number increases with the age of the thallus, so that in the lower portions they may fill up all the spaces between the longitudinal filaments, making a very solid central tissue.

In the cells of both cortex and medulla will be seen many small granules of **fucosan**, often associated with the plastids. Their chemical nature is uncertain but there is probably a mixture of tannins and polysaccharides present. They do not stain with Iodine but are coloured bright red by a solution of Vanillin in Hydrochloric acid.

> Cut a longitudinal section through the apex of a frond, running across the small groove which can be seen at the extreme tip. Stain, and mount in Lactophenol.

In such a section taken through the growing apex it will be possible to see one of the large apical cells lying in a groove at the top of the branch. These apical cells are roughly triangular in shape with a blunt apex upwards. There is a short row of them across the width of the frond. They cut off cells from all three sides. As a result of active growth of the cells thus cut off, the apex itself remains buried in a depression, whereby it is afforded some protection. All growth in *Fucus* is dependent on the divisions of the apical cells. Branching is effected by the division of the group of apical cells into two.

> *Reproduction : Male Conceptacle.*—Cut a transverse section of a male plant in the fertile region ; stain in Cotton Blue and mount in Lactophenol.

The conceptacles are large and open to the exterior by a median **ostiole.** Through this ostiole, hair-like **paraphyses** project to the exterior, whereby they partly close and protect the ostiole. The wall of the conceptacle is lined with a small-celled tissue which is continuous with the external surface. This is brought about by the fact that the conceptacle originates as a slight depression in the surface of the thallus, which bears a hair. After the disappearance of the hair the cavity increases in size, becoming more and more sunk into the tissues of the thallus, until much of it is lying in the medulla. The antheridia are borne partly on highly complex branched hairs, which arise from the wall of the conceptacle, and also from short filaments which arise directly from the conceptacle. Paraphyses are also developed as sterile branches of the antheridial hairs. Each antheridium is a stalked, unicellular body, oval in shape, with a double wall. Inside, at maturity, are numerous **antherozoids** which are small and pear-shaped, with two flagella, one pointing forwards and the other backwards. Each has also a single, bright orange-coloured plastid. The inner surface of the conceptacle may develop ridges whereby the surface area over which the antheridial branches can be developed is increased.

> *Reproduction: Female Conceptacle.*—Cut a section through the fertile region of a female plant showing conceptacles. Stain in Cotton Blue and mount in Lactophenol.

In *F. vesiculosus* and *F. serratus* the sexes are produced on different plants. In the allied species *F. spiralis*, however, the conceptacles are hermaphrodite, both types of sex organs occurring in the same conceptacle.

The development and structure of the female conceptacle is the same as that of the male, but the internal organs are quite distinct. The unbranched **paraphyses** arise mainly from the floor of the conceptacle, and the floor itself is not thrown into folds. Each **oogonium** is borne on a single basal cell and consists of a large, swollen, thick-walled body, the wall of which consists of three distinct layers. The inner body is densely granular and pigmented, and in older oogonia it can be seen to have divided up into a number of segments, actually eight, though rarely more than six can be seen at once. These are the **oospheres,** and when mature they are released in a packet by the bursting of the outer membrane, the **exochiton.** In this condition they escape from the conceptacle into the sea water and are later released as isolated spherical bodies by the bursting of the **mesochiton** and the solution of the innermost wall or **endochiton.**

> *Reproduction: Fertilization.*—Squeeze some ripe oospheres out of a female conceptacle into a drop of sea water and add some ripe antherozoids squeezed from the tip of a male head. Examine under the high power.

The antherozoids will be seen to be active and will soon seek out the oospheres, which can be distinguished by their large spherical shape. Those oospheres to which the antherozoids have become attached can be recognized by their active rotating movement. This continues till finally one of the antherozoids penetrates the oosphere and its nucleus fuses with that of the oosphere. This causes all the others to leave, due presumably to the secretion of a toxic substance. A wall is secreted around the zygote so formed and an **oospore** is the result.

The whole process only takes a few minutes and can be easily followed under the microscope, provided that really fresh material is used and that it is mounted in fresh sea water.

Section 2. Fungi

Preparation of Agar.

For the cultivation of many Fungi it is necessary to grow them upon an artificial medium of known composition. This medium may be liquid or solid, but for most fungal work a solid medium will be found most convenient. Agar, obtained from the dried thallus of the Red Alga, *Gelidium corneum*, is collected in Japan, and dried in large quantities for this purpose. Gelatine is sometimes used, though more in bacteriology than in mycology. About 2 per cent. of Agar, or 10 to 15 per cent. of Gelatine, are suitable concentrations.

The other ingredients of the medium may be either synthetic or natural.[1] Among the latter, malt extract, dung, Corn, Pea or Bean meals, Prunes, Potatoes and plant decoctions are most commonly employed. In each case a solution is obtained by boiling about 5 per cent. of the substance with water. To this 2 per cent. Agar is added. If the solution is turbid it is best filtered before the Agar is added. The solution with the added Agar is then placed in a steamer and heated. Under these conditions the Agar slowly dissolves, and a solution is obtained which solidifies at room temperature.

Sterilization of the tubes is most conveniently done after they have been filled with Agar. Using test-tubes 6 in. by ⅝ in., of the rimless bacteriological type for preference, about 10 c.c. of the melted Agar is placed in each and the tube closed with a cotton-wool plug. It is now necessary to sterilize the tubes. This can be best done by heating for fifteen to twenty minutes at a temperature of 115° C. in an autoclave or steam pressure cooker. Where this is impossible, satisfactory results will be obtained by the more tedious process of heating in boiling water for twenty minutes on three successive days.

After sterilization the tubes should be laid out in rows, the tops resting on a length of thick glass tubing. In this way a long slanting surface of the medium is obtained, which gives a greater surface on which the organisms may grow.

Inoculation of the tubes is done by first heating to redness a Platinum wire and allowing it to cool without touching any surface, then gently removing the cotton-wool plug and passing the top of the tube through a flame. A small quantity of the material to be cultured is then introduced into the tube with the Platinum wire, after which the rim of the tube is again passed through the flame and the cotton-wool plug replaced. Great care should be taken that the cotton-wool plug does not come into contact with anything during the process. It should be held by the top in the fingers while the inoculation is being done.

Most Fungi will grow well enough at room temperature. Many Bacteria will grow better in an incubator at a temperature of 37° C.

A. ARCHIMYCETES

1. ***Plasmodiophora brassicae.*** *Vegetative Structure.*—Transverse sections of a young infected cabbage root stained in Iron Alum Hæmatoxylin and mounted in Balsam; or stained in Cotton Blue and mounted in Lactophenol.

Plasmodiophora brassicae occurs in the roots of Cabbages and other Crucifers, and early stages of the Fungus are found in sections of young infected seedlings a few weeks old.

The **myxamoebae** are at first spindle-shaped and uninucleate, but as nuclear multiplication occurs the myxamoebae become rounded and are regarded as **plasmodia.** These plasmodia are devoid of any cell wall; one or more may occur in the host cell, and they tend to aggregate together to form a single structure. The protoplasm is granular and stains fairly deeply. Numerous nuclei are to be found scattered in the protoplasm. Each nucleus is enclosed in a very delicate nuclear membrane, and contains a central spherical **karyosome** which stains deeply. Stages in the nuclear division are rarely found, and all the nuclei in a single plasmodium divide simultaneously, so that only one stage is likely to be seen in any particular plasmodium.

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[1] For receipts see Appendix.

Occasionally a membrane may be secreted around a plasmodium, whereby the plasmodium becomes isolated in a host cell. Apparently the wall of the **cyst** thus formed is later reabsorbed, and the plasmodium can continue development.

The host cell will be stimulated to division by the presence of the plasmodium, and its nucleus is often greatly enlarged. Giant mitotic figures in the host cells are therefore sometimes met with.

> *Reproduction and Spore Formation.*—Transverse sections of old infected roots of Cabbage plants stained in Cotton Blue and mounted in Lacto-phenol.

A meiosis occurs before **spore** formation, and in favourable material the stages in this process may be found in sections containing spores. These spores are not arranged in a definite pattern as they are in certain allied genera, but are packed fairly closely together, filling either a part or the whole of the host cell. When each spore is separated it will be seen to be a small spherical body with a thin hyaline membrane. Its nucleus is not easily recognizable.

Germination of the Swarm Spores.—If some spores are isolated and placed in a hanging drop made of an infusion of Cabbage roots they may be induced to germinate. The wall splits and a single pyriform **swarm cell** emerges. This cell is devoid of any recognizable cell wall. It may be fixed on the slide with fumes of Osmic acid. Each swarm cell contains a single nucleus and is provided with two flagella attached to the apical end. One flagellum is long and easily seen, while the other is very short and difficult to make out. It is these bodies which reinfect the cabbage seedlings, entry being through the root-hairs.

Stages in the further development of the swarm cells are very difficult to observe. If an infusion of spores in water is introduced among germinating cabbage seeds the penetration of the swarm cells into the root-hairs may be observed. After entry each swarm cell becomes amoeboid and then secretes around itself a thin wall. The contents then divide into a number of very small **gametes** which are finally liberated.

> 2. ***Fuligo septica*** or other species. The study of the plasmodium of a Myxomycete presents difficulties because it is extremely delicate and cannot be easily handled. The most satisfactory method is to find a plasmodium developing in the field and try and induce a portion to grow over a microscope side placed beside it. The portion of

the plasmodium on the slide can then be separated, fixed with Bouin's Fluid and subsequently stained with Iron Alum Haematoxylin. Cultures can also be made from germinated spores. (See below.)

The cytoplasm is granular but numerous thickened, branched strands permeate it, especially in the older portions. In a young marginal portion of the **plasmodium** one may recognize extended processes or **pseudopodia,** which consist of fine granular protoplasm with little differentiation. In the older portions we find the thicker and tougher **ectoplasm.**

The nuclei are small and spherical with a deeply staining **karyosome** in the centre. All nuclei of a plasmodium divide at the same time and in a large plasmodium there may be many thousands of nuclei. In *Fuligo septica* the plasmodium is bright yellow but the colour varies in other genera. It is frequently white, but it may be red, yellow, or orange. The volume of a mature plasmodium also varies. In *Fuligo septica*, which is one of the largest, it may consist of as much as a litre of almost pure protoplasm.

3. ***Arcyria denudata.*** Select a mature sporangium and immerse it in absolute Alcohol for a few moments to remove the air. Mount in dilute Glycerine and tease out gently with needles.

The stalk of the sporangium is thick and solid, composed of amorphous material of a horny consistency. The stalk enlarges upwards and unites with the wall of the **sporangium.** Within this the spores are formed together with the **capillitium.** The latter consists of a mass of elastic filaments, marked irregularly with transverse striations, which branch and anastomose in a very complex manner.

The **spores** lie scattered among these capillitial threads, each spore being spherical in shape with a slightly warted wall. If the sporangium has been treated with absolute Alcohol some of the spores may be collapsed.

The spores are shed after the bursting of the thin wall of the sporangium, due apparently to the expansion of the capillitium resulting from the twisting and turning of the capillitial threads under changes of atmospheric moisture.

Other Myxomycete sporangia may be studied but the details of construction will vary greatly in the different genera, more particularly in the form of the capillitium. For a detailed description of the sporangial structure of the different genera, the student is referred to the "Guide to the British Mycetozoa" by G. Lister, published by the British Museum, South Kensington, London.

4. ***Reticularia.*** *Germination of the Spores.*—The spores of species of the Myxomycetes can generally be induced to germinate in a sucrose solution kept in the dark. The time taken varies according to the species employed and the age of the material. Although in *Reticularia lycoperdon* the spores retain their vitality for a number of years, the same is not true of all genera, and generally the older the spores the longer they take to germinate.

The following table gives an indication of the time which may be expected to elapse between the sowing of the spores in a watch-glass of the sugar solution of appropriate strength and the appearance of a good percentage of germination :—

TABLE OF MYXOMYCETE SPORE GERMINATION

Species Employed.	Strength of Sucrose.	Percentage of Germination.	Time Required.
Reticularia lycoperdon . . .	2·0	80	90 min.
Stemonitis splendens . . .	2·0	50	24 hrs.
Fuligo septica	2·0	10	24 hrs.
Lycogala epidendrum . . .	1·0	70	70 hrs.
Trichia varia	1·0	25	5 days

Samples may be taken out and placed in hanging drops for observation. If the cover-glass with the spore suspension is allowed to stand until the liquid is nearly but not completely dry and is then flooded with Bouin's Fluid, the swarm cells become fixed to the cover-glass. Thereafter they can be stained with Iron Alum Haematoxylin and mounted in Balsam in the usual way.

The swarm cells have two, unequal, apical flagella ; they are pyriform in shape with a single nucleus with a well-marked karyosome. After a while the flagella are retracted and the swarm cell becomes amoeboid. Further development can sometimes be induced to take place by germinating the spores in a Petri dish containing Knop Agar, but it is not easy to prevent the growth of contaminants, which swamp the development of the Myxomycete plasmodium. The purification of such cultures is outside the scope of most students. Further particulars are given by Cayley (*Trans. Brit. Mycol. Soc.*, vol. xiv., 1929).

B. PHYCOMYCETES

1. **Mucor.** *Vegetative Structure and Asexual Reproduction.*—Material may be grown on agar or may be obtained from a spontaneous culture on bread kept damp for a few days under a bell jar. Various other interesting species may be obtained by sowing soil particles on a suitable agar substratum. Malt agar is quite suitable.

Plunge a small piece of the material in strong Alcohol to remove the adherent air ; stain in Cotton Blue and mount in Lactophenol.

The fungus consists of a **mycelium** made up of numerous **hyphae** without cross walls. These hyphae are branched, and together form the interwoven mycelium. Arising from the mycelium are numerous vertical filaments, the **sporangiophores,** which bear sporangia at the apices.

The **sporangium** consists of a thin-walled spherical sac which is filled with oval, coloured or colourless spores. In the centre of the sporangium arises a central **columella,** varying in shape in different species, whose function is to assist in spore dissemination. When the sporangium is mature, water is absorbed by the sporangium, which ruptures somewhere near its base and finally dehisces, leaving a small part of the original wall as a frill around the base of the columella. The spores are partly scattered and partly held in a drop of moisture which collects on the apex of the columella, and when this dries up they may be scattered in the wind, together with the remnants of the sporangium wall.

Various stages in the development of the sporangium will be seen in mounts taken from a culture, and a number of different species may be met with on bread. It is by no means easy to distinguish between them. Some have sporangia borne singly on a stalk, the *Mucor mucedo* type, others have branched sporangiophores on which sporangia of different ages are developed. In *Rhizopus nigricans* (=*Mucor stolonifer*) the sporangiophores arise in pairs or groups, each forming a single apical sporangium. This fungus may also be distinguished by the formation of a richly branched system of stolons or thickened hyphae, impervious to Cotton Blue and generally brown in colour, which is formed in the substratum. It is from this that the sporangiophores arise. This fungus is very common and is frequently mistaken for a typical species of *Mucor*.

> *Sexual Reproduction.—Mucor* exhibits heterothallism, and it is very rare for sexual reproduction to appear except under specially controlled conditions. If cultures of authenticated plus and minus strains are available sexual reproduction may be easily obtained. Sterilize a Petri dish, pour out about $\frac{1}{8}$ in. of melted agar in the bottom, and when set solid inoculate it with the two strains, one on each side of the plate. As the mycelia of the two cultures grow they will spread out in all directions from the point of inoculation and will finally come into contact in the middle of the dish. Along the line of meeting of the plus and minus strains a line of black zygospores will be formed.
>
> Take a few hyphae from this zone, mount in Lactophenol and examine under the low power. Various stages in sexual reproduction of the fungus can be seen. It is unlikely that any early stages will be found prior to the fusion of the gametangia together, since they would be separated from one another by mounting.

At an early stage the **gametangia** can be seen as two enlarged club-shaped bodies, cut off from the hyphae by cross walls and united to one another along a line which is clearly visible. They are pale brown in colour. At a later stage the colour becomes dark brown, the gametangia have swollen and the line separating them has disappeared, so that the whole becomes a spherical body. At this stage fusion of the nuclei is believed to have taken place, and this is followed by the further thickening of the wall of the zygote, which develops into a thick-walled resting **zygospore.** In *Rhizopus nigricans* the process is essentially similar, but in allied genera it can be seen that the zygote is formed chiefly from one of the component gametes which is regarded as the female, the male gametangium withering away after fertilization has been achieved.

> 2. **Saprolegnia.** *Cultivation and Asexual Reproduction.*—Species of *Saprolegnia* may be readily obtained by immersing hemp seeds cut in half, in samples of pond water. The fungus is also developed on "ant eggs," dead flies and similar bait placed in shallow vessels of pond water. The fungus appears in about two days, and by the fifth or sixth day, at laboratory temperatures, the asexual reproductive organs begin to appear. Sexual reproduction appears in the same culture slightly later, at the end of nine or ten days. Rough cultures obtained in this way may be purified by snipping off a few hyphae and placing them in a dish of 2 per cent. corn-meal agar, on which the fungus grows readily. The cultures may be further purified by cutting off a few hyphae from this culture, with sterilized instruments, and placing on a second plate of agar. If a portion of this agar containing the fungus is placed in a dish of water with a few hemp seeds a pure culture of the fungus will be obtained which will last for a considerable time. Mount a few hyphae in water and examine under the low power.

The mycelium is composed of non-septate hyphae which are somewhat branched. The contents are dense, particularly near the apices of the hyphae, which are cut off by a wall and constitute the **zoosporangia.** Each zoosporangium, when mature, opens by an apical pore and the **zoospores** escape singly, one after another, separate at once and begin to swim about. This is an important difference between the genus *Saprolegnia* and the allied genus *Achlya*, in which the zoospores cluster around the apex of the zoosporangium.

The zoospores are small, pip-shaped bodies containing very little visible food reserves, though several tiny oil globules can be made out. After a while they withdraw their pair of apical flagella and undergo a period of rest as round non-motile bodies, closely resembling the zoospores as far as internal contents are concerned. At length the morphology of the body changes and they may be found some twenty-four hours later as kidney-shaped structures with a pair of lateral flagella. In this state they finally come to rest on a suitable substratum, which in nature is a piece of decaying wood, and there grow into a fresh filament. This double swimming stage is termed **diplanetism.**

> *Sexual Reproduction.*—Mount some older material in water and examine under the high power. Either *Saprolegnia* or *Achlya* can be used.

The **oogonia** will be seen as spherical or ellipsoidal bodies containing a number of **oospheres** (Fig. 10). The oogonia may be either terminal or intercalary, either on the main hyphae or on lateral branches from them. The wall of the oogonium is pitted and relatively thin. The oospheres have thin walls and contain considerable reserves of oil which, by the time they are mature, become combined into a single globular body, which may be formed centrally (centric) or at the margin (excentric) of the oosphere.

The **antheridia** are not always functional, and are entirely absent in some species. When present they may be found either on the same (androgynous) or on different (diclinous) hyphae from the oogonium. A separate antheridium may be employed to fertilize each oosphere, though complete fertilization rarely occurs and a number usually develop parthenogenetically. Each antheridium is a long, slender, somewhat coiled structure, and it is cut off by a septum from the hypha which bears it. It is applied laterally, not apically, to the wall of the oogonium, and a fertilization tube can be seen passing through a pit in the wall of the oogonium and penetrating the oosphere. The fertilization tube is very fine and staining in Cotton Blue will help to make it visible.

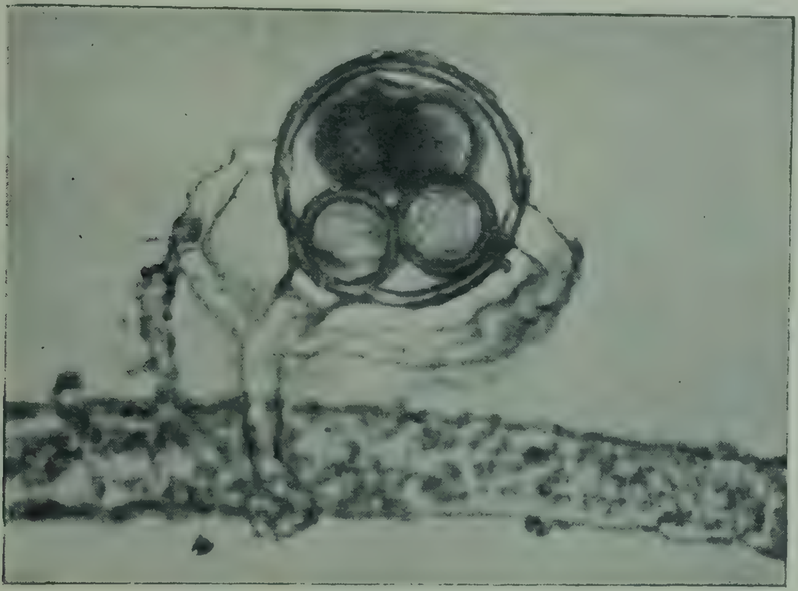

Fig. 10.—*Achlya colorata.* Oogonium with associated antheridia.

The **oospores** differ but little from the oospheres, except that their walls are thicker and the oil drop more completely organized. It is rare to find an oogonium with only some of the oospheres developing.

This description must necessarily be very general, for the detail varies much in different species. In some the oogonia are spiny, and in others the antheridia arise from the base of the oogonium, but such differences do not affect the main points which are given above.

3. ***Monoblepharis sphaerica*** or other species : *Morphology.*—Species of *Monoblepharis* are common in ponds and slow-flowing rivers all over Britain, but they are difficult to find. Collections should be made of rotten sticks of almost any tree, which have remained submerged until they are completely water-logged, but have not lost their bark. If a number of these are brought into the laboratory, broken into short

lengths, immersed in distilled water and left in a refrigerator working at about 2° C. above freezing point for a few weeks, it will be found that, on returning them to room temperature a copious growth of whitish-grey, translucent filaments develops, especially around the lenticels.

The hyphae of *Monoblepharis* are easily recognizable under the microscope because of the curious net-like form of the cytoplasm. This curious net-like system of the protoplasm is unlike that found in any other Fungus and this, together with the uniform grey colour, makes them easily recognizable. The hyphae rarely branch and are coenocytic, with a very few septa occasionally formed in old coenocytes. The wall of the hypha is thin and greyish-white in colour but the hyphae themselves are relatively thick and easily seen under the low power.

Asexual Reproduction.—Mount a few young tufts of the fungus in water. Tint, if desired, in aqueous Cotton Blue.

The **zoosporangia** are long and narrow, little, if any, wider than the vegetative hyphae. They are formed terminally on hyphae, from which they are cut off by a septum. **Zoospore** differentiation begins at the apical end of the zoosporangium and the zoospores are usually produced in a single file. Each zoospore contains a clearly defined nucleus.

The zoospores are liberated by the dissolution of the apex of the zoosporangium and they emerge in a single file, one after another. Each zoospore possesses a single apical flagellum and is roughly spherical in shape. Frequently some zoospores fail to emerge and under these conditions they may germinate in the zoosporangium by the production by each of a hypha, which may penetrate through the wall of the zoosporangium.

Sexual Reproduction.—Mount some older material in Lactophenol, after staining lightly with Cotton Blue.

The structure of the sex organs will vary considerably according to the species being examined, but they fall into two main types.

The sex organs consist of **antheridia** and **oogonia,** formed on hyphal branches. The antheridium may be developed on the wall of the oogonium, but in the majority of species it originates at the apex of the branch, being subsequently pushed sideways by the enlargement of the oogonium. In a few species it is formed below the oogonium. The antheridium is a short tubular cell which is cut off by a septum. Its contents divide up into about half a dozen **antherozoids** which escape through an apical pore. Each antherozoid is more or less spherical and may crawl out of the antheridium by amoeboid movement. It possesses a single trailing **flagellum.**

The oogonium contains a single spherical **oosphere** with a thin yellowish wall. This oosphere may either remain within the oogonium or it may be extruded, so as to sit in a cup at the apex of the oogonium. After fertilization an **oospore** is formed, with a thick golden-brown wall which in some species is beset with large warts.

4. **Pythium.** *Cultivation and Asexual Reproduction.*—Many species of *Pythium* occur in the soil, and probably the best method of studying the fungus is to obtain a pure culture in the way described for *Saprolegnia*. Some species, like *Pythium debaryanum*, cause the damping-off of seedlings, and a copious growth of the fungus can generally be obtained by keeping a very dense growth of cress seedlings moist and warm under a bell jar. If seedlings are removed, stained in Cotton Blue and mounted in Lactophenol, by carefully squashing out the hypocotyl it will be possible to see the fungus spreading among the cells of the seedling, causing a tissue breakdown. The hyphae are non-septate, much finer than those of *Saprolegnia*, and occur both inside and outside the host tissue.

To study the reproductive organs of the fungus a culture is best employed. The **zoosporangia** are formed first and appear as small oval bodies borne terminally on the hyphae. It is often difficult to induce the formation of zoospores, but this can be done in a hanging drop. A small ring of glass about 1 mm. high is cemented on to a slide and allowed to dry. The hyphae containing zoosporangia are immersed in a drop of water on a cover-glass which is then inverted on the glass ring, and the edges sealed with Vaseline. In this way a drop of water comes to hang in a tiny moist chamber between the slide and the cover-glass. Under such conditions the zoosporangium may be seen to produce a vesicle on one side into which its contents pass. In the vesicle about fifteen zoospores are differentiated. Each is kidney-shaped with two almost equal flagella inserted on one side.

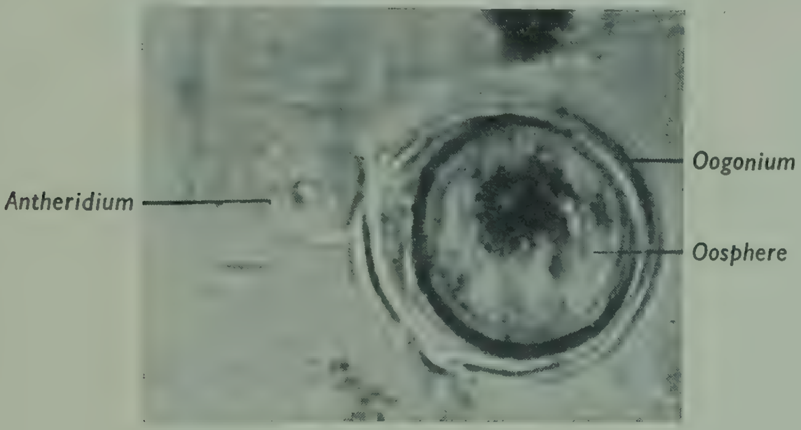

FIG. 11.—*Pythium debaryanum.* Oogonium with antheridium.

These zoospores finally swim away, and on coming to rest withdraw their flagella and produce new hyphae.

Sexual Reproduction.—Material showing sexual reproduction can easily be obtained from pure cultures, though occasionally it may be found in parasitized material. Stain with Cotton Blue and mount in Lactophenol.

The **oogonium** is spherical and thin-walled (Fig. 11). It contains a single **oosphere** whose wall is almost adherent to that of the oogonium. The

antheridium may be either diclinous or androgynous, arising as a narrow hypha which cuts off a terminal antheridium. Fertilization is less easily seen than in *Saprolegnia*, though the antheridium is laterally applied and penetration is effected by a fine tube. The **oospore** formed as a result of fertilization has a single thick wall and contains much reserve material. The structure of both the oogonium and oospore varies in different species. In some the oogonia are spiny, in others they are smooth.

> 5. **Cystopus.** *Pathological Effect and Asexual Reproduction.*—The species *Cystopus candidus* is found most commonly on the stem and leaves of *Capsella bursa-pastoris*, the Shepherd's Purse, where it causes what is known as "White Rust." Cut a transverse section through an infected leaf and examine the asexual reproductive organs.

The reproductive organs develop below the epidermis, with the result that when they are mature the epidermis is split, the lesion leaving the asexual spores exposed. The reproductive organs themselves will be seen to consist of numerous short hyphae rising side by side on the base of the cavity, from which chains of round spores are cut off. In fairly young material the spores remain undisturbed, but in slightly older examples the terminal spores have already separated and only the younger spores remain, attached to the basal hyphae. These hyphae are regarded as **conidiophores,** comparable with the branched structures in *Peronospora*, while the small spherical spores are **conidiospores,** similar to the oval spores of *Peronospora*. Each lesion produces a very large number of spores. Unlike *Peronospora*, however, these spores give rise to **zoospores** on germination, and are therefore correctly regarded as sporangia, borne on sporangiophores.

If the passage of the mycelium through the host tissues is examined in sections stained with Cotton Blue and mounted in Lactophenol, it will be seen that the hyphae are intercellular, only **haustoria** passing into the cells. These haustoria are quite short and knob-like and contrast clearly with those of *Peronospora*.

> *Sexual Reproduction.*—The sex organs are developed deep in the tissues of the host plant as well as in the cortex, and in heavily infected material almost all the tissues except the vascular bundles become infected.

The mycelium being intercellular, the sex organs are developed in the intercellular spaces, and with heavy infection the host tissue becomes so disorganized that it is difficult to make out which are the cells and which the spaces between them.

It is not easy to interpret the early stages in the development of the sex organs, but in mature material **oogonia** may be found containing a single **oosphere** (Fig. 12). At this stage the oosphere may still have a thin wall, and is considerably smaller than the oogonium which encloses it.

After fertilization the **oospore** is much more easily seen. On the outside is a very thick **epispore** which appears almost double, its outer surface being covered with wart-like outgrowths. The inner layer consists of a thin **endospore.** In stained material it is sometimes possible to make out the numerous nuclei. On germination the contents of the oospore divide up into a large number of **zoospores,** which escape into a vesicle developed through a split in the thick wall of the oospore.

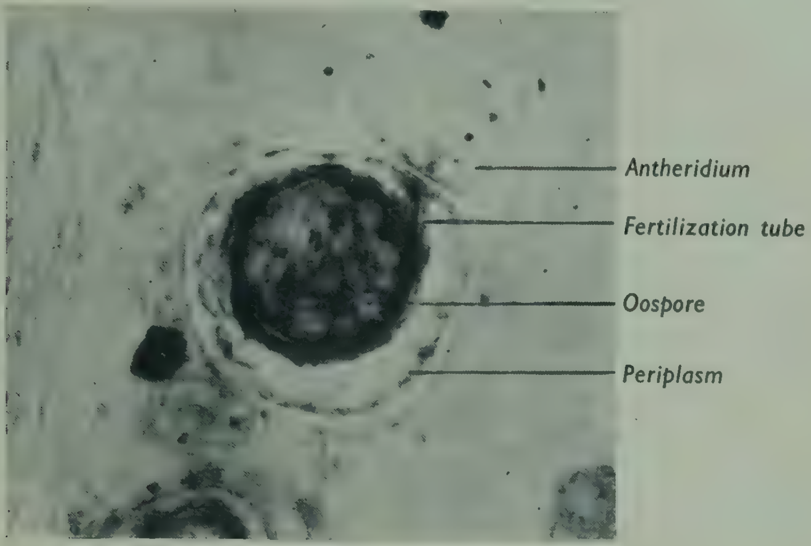

FIG. 12.—*Cystopus candidus.* Mature oospore.

6. **Peronospora.** *Pathological Effect and Asexual Reproduction.*—Species of *Peronospora* attack a large variety of flowering plants. Suitable material can generally be obtained from diseased plants of *Capsella* or *Arabis.* The fungus appears as a white patch on the leaves and stems, sometimes causing slight swelling or twisting of the latter. Externally this fungus may be easily mistaken for the last example, *Cystopus candidus,* though in their structure the two can be easily distinguished. The chief naked-eye distinction is the powdery appearance as contrasted with the smooth surface of the pustules in *Cystopus.*

To see the asexual reproductive organs, sections should be cut through the leaves, but care must be taken not to crush them or the fine fungal tissue will be damaged. The quality of the material will usually be improved if fresh material is left for a few hours in a damp atmosphere, *e.g.,* shut in a tin, which encourages the formation of conidiophores.

If a good thin section is obtained it will be seen that the hyphae bearing the reproductive organs pass out through the stomata. They are at first simple structures, which soon branch dichotomously several times, forming a small tree-like body, the **conidiophore.** The tips are prolonged into a series of fine filaments from the ends of which large oval or nearly spherical **conidiospores** or **conidia** are produced.

These spores are shed, and on germination give rise to a germ tube which re-enters the host tissue, producing a fresh infection.

Deeper in the tissue of the leaf the mycelium may be seen, especially in sections which have been stained with Cotton Blue and mounted in Lactophenol. The hyphae of this mycelium pass between the host cells, never through them, but they give off fine **haustoria,** which are able to penetrate the cell walls and may be seen as complexly branched structures within the host cells. It is by means of these haustoria that the fungus obtains its food supply.

> *Sexual Reproduction.* The sexual reproductive organs are usually found in the tissues of the stem, frequently in the pith, though in some species they may develop in the cortex. Cut sections across an infected stem and examine under the high power.

The **oospores** will be seen as spherical bodies lying freely in cavities between the host cells. Each oospore has a thick wall whose outer surface is warted or reticulate, with a number of short spines projecting from it. In good material it may be possible to see the stalk of the oogonium appearing as a thin hypha attached to one end of the oospore.

It is unlikely that early stages showing the oogonium and antheridium can be made out, for these organs look very much like the vegetative hyphae, and without specially cut and stained microtome sections they cannot be easily discovered.

C. ASCOMYCETES

> 1. ***Aspergillus.*** *Structure and Reproduction.*—Stain a few filaments of the fungus in Cotton Blue and mount in Lactophenol.

The hyphae will be found to be divided by **septa** into separate cells, contrasting sharply with the coenocytic hyphae of the Phycomycetes. Certain large, straight hyphae grow up vertically with apices spherically enlarged to form **oedocephala,** from which more or less spherical **conidia** are abstricted in chains forming a kind of halo around the oedocephalum. The youngest conidia lie at the base of the chain and are slightly smaller than the terminal ones and possess a somewhat less well-developed and smoother wall. At the base of each chain is a projection of the oedocephalum, the **sterigma** from which the conidia are formed.

On liberation these conidia germinate by the development of a hypha, a process which may sometimes be observed on the slide.

This asexual reproduction is the usual method of reproduction in *Aspergillus*, for sexual reproduction is rare, and occurs only under exceptional conditions. In this process two hyphae become associated together, one, the **antheridium,** coiling around the other, the **oogonium,** after which additional hyphae grow up from the base and enclose the sex organs within a bright-coloured wall, the **peridium.** The complete structure is regarded as a **perithecium** similar to that found in *Erysiphe.* Subsequently, a number of **asci** are formed which liberate **ascospores.** These latter stages can only be observed in sections, and it is unlikely that the student will have the opportunity of studying stages in the sexual reproduction of *Aspergillus* for himself.

The mature perithecia, however, are easily perceived as bright yellow granules among the hyphae in an old culture. If one of them is crushed under a cover-slip the asci and ascospores may be seen.

2. ***Erysiphe.*** *Pathological Effect and Asexual Reproduction.*—Species of the Erysiphaceae occur on the leaves of many flowering plants, and all agree in the main points, differing chiefly in the number of asci produced in the perithecium and in the shape of the appendages to the perithecia.

If a section of a leaf attacked by one of these fungi is examined during the summer months it will be possible to see the asexual reproductive organs which are developed at that time of year.

The **mycelium** of the fungus is developed on the surface of the leaf, **haustoria** passing down into the epidermal cells, but the lower tissues of the leaf are unaffected. The asexual reproductive organs consist of vertically growing **conidiophores,** which are simple unbranched hyphae which abstrict oval **conidiospores** in chains from their upper ends. On liberation these conidiospores germinate by the production of a hypha which forms a fresh mycelium on the same leaf or on another, depending on where the spore has fallen.

Sexual Reproduction.—Scrape off some of the mycelium from an infected leaf on which the perithecia can be seen with a lens, and mount on a slide in dilute Glycerine.

The **perithecia** are brown in colour, becoming darker with age. The outer surface is warted and bears numerous long filamentous **appendages,** the shape of which depends on the genus. In *E. graminis* they are rather short and straight. Towards the lower surface of the spherical perithecium short, thick, peg-like appendages may also be found. As has been previously pointed out, the size and shape of these appendages vary, and consequently afford a good method of identification of the genera.

Squash a single perithecium and observe that it consists of a dark coat, the **peridium,** two cells thick, enclosing a number of **asci** which escape through the opening. These asci are elongated sac-like bodies with a colourless, delicate wall (Fig. 13). There are a number enclosed in the perithecium in *Erysiphe*, but only one in the allied genus *Sphaerotheca.*

Except under favourable conditions it is unlikely that the ascus will have divided up into the eight **ascospores,** for this takes place considerably later, when most of the perithecia have been blown or washed off the leaf. Good material may sometimes be obtained by collecting dried leaves which have already been shed.

The arrangement of the asci in the perithecium can be seen by cutting a transverse section through a heavily infected leaf. In this way some perithecia will be cut longitudinally. It will be seen that the perithecium arises from a basal tissue, and that the wall is made up of a dark outer layer and a colourless inner layer. Usually three asci will be seen in any one section.

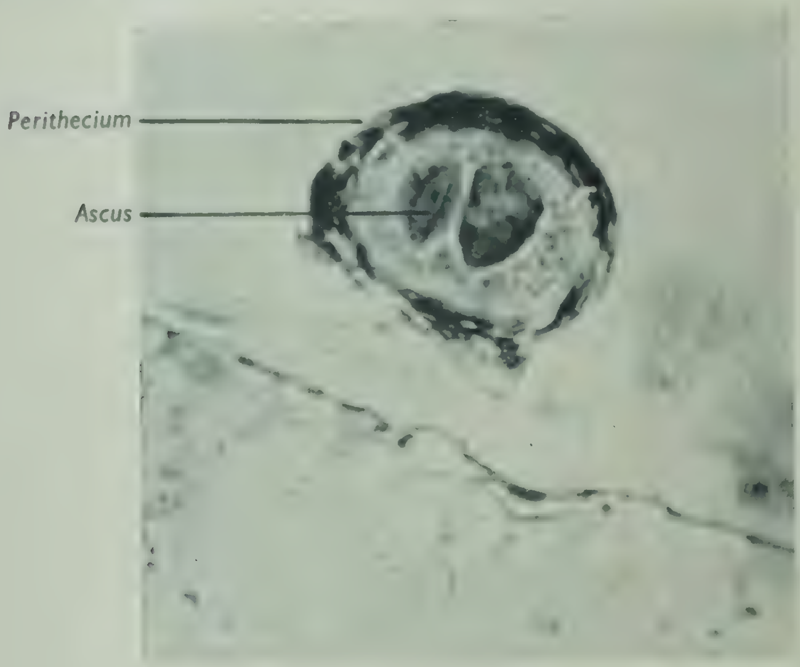

FIG. 13.—*Erysiphe cichoracearum.* Vertical section through perithecium.

3. **Peziza.** *External Morphology.*—The fruiting body is generally cup-shaped, coloured on the inside and colourless outside.

The colour varies with the species, usually orange, brown or red. In *Peziza aurantia*, as the name suggests, it is bright orange, and the fruiting body is one of the largest species occurring in this country. This species may be collected in summer and autumn growing in mixed woodlands on humus on the ground. If examined in the field it is sometimes possible to observe a whitish cloud arising from the fruiting body, which consists of the **ascospores,** forcibly expelled from the **asci,** which are immersed in the tissues of the inner surface of the cup.

The base of the cup may develop a short root-like portion which passes imperceptibly into the fine white mycelium. Some species have a definite stalk upon which the cup is borne, but most of the larger species are sessile. They may be found singly or in groups.

Section through Apothecium.—Cut a longitudinal section through part of a mature fruiting body. Stain in Cotton Blue and mount in Lactophenol.

On the outside the cells are relatively large, forming a **pseudoparenchyma,** and bear numerous brown appendages or hairs. The inner tissues consist of much smaller cells, comprising interwoven septate hyphae with large air spaces between them. The innermost layer, the **hymenium,** stains more deeply and produces long septate **paraphyses** among which the **asci** arise.

Each ascus is a very long, narrow, tubular sac which opens at the upper end by a lid or **operculum.** It contains eight oval **ascospores** which are arranged obliquely along the ascus. Each ascospore has a thin, smooth wall and sometimes appears to be binucleate. The spores when ripe are forcibly ejected through the operculum. In certain species the outer wall of the ascospores may be sculptured with very complex patterns.

4. ***Rhytisma acerinum.*** *External morphology.*—The Fungus attacks the leaves of the Sycamore (*Acer pseudoplatanus*) forming large black patches. Examine one of these infected leaves with a hand lens.

The black **stroma** is developed chiefly in the tissues of the upper surface of the leaf, the lower surface being little affected. The patches of diseased tissue are at first greenish yellow in colour, but turn brown and finally black as they mature. Young stromata may be not more than a quarter of an inch across but the fully grown ones may reach an inch in diameter.

Asexual Reproduction.—Cut a transverse section through a young stroma in the tissues of the leaf, stain in Cotton Blue and mount in Lactophenol.

Only the upper epidermal cells of the host are attacked, the fungal mycelium forming a group of hyphae in the base of each cell, from which vertical hyphae grow up towards the outer surface of the cells. Dark staining material is deposited on the cell walls, especially on the lower tangential ones.

At a later stage, the number of vertical hyphae increases and black material is deposited on the radial walls. Finally the radial walls of the epidermal cells are ruptured and the outer tangential walls are pushed up by the development of vertical **conidiophores** which abstrict chains of small, spherical **conidiospores.** Meanwhile, the lower portions of the epidermal cells become disorganized and filled with black material in which the fungal mycelium becomes embedded.

The little pustules, in which these conidia are developing, enlarge and may unite with adjacent ones forming an **acervulus.**

Sexual Reproduction.—Cut a transverse section through a mature stroma. Stain in Cotton Blue and mount in Lactophenol.

The stage in the development of the apothecium will depend upon its age and it may be desirable to cut sections from several leaves to obtain different stages.

The **apothecium** rests on a basal mass of fungal material of considerable thickness in which it is difficult to make out the separate hyphae. From this tissue vertical hyphae grow up and from their apices **asci** are produced.

Each ascus is an elongated cell, broader at its apex and narrow at its base. The **ascospores** are long and filiform and are often somewhat twisted around one another.

The apothecial cavity is lined below by a **hypothecium** composed of black thick-walled fungal tissue covering the remains of the lower surface of the epidermal cells. Above, the apothecium is covered over by an **epithecium** composed of thin-walled fungal cells rather loosely associated together. When the asci are ripe, changes in the apothecium cause the opening of the epithecium to liberate the spores, but at no stage does the apothecium open very widely.

5. ***Saccharomyces.*** *Structure and Budding.*—Brewer's yeast, *Saccharomyces cerevisiae*, may be induced to grow actively by placing a small quantity of the pressed yeast in a flask containing about 5 per cent. of sucrose and keeping warm overnight at a temperature of 25° to 30° C.

Take a small drop of the solution and mix with a drop of water on a slide. If good results are to be obtained the liquid should be well diluted so that the individual cells can be observed. Stained slides can be obtained by allowing the drop to dry up, fixing with Osmic acid vapour and then staining with 1 per cent. Cotton Blue or Acid Fuchsine for just under one minute. Wash and mount in Glycerine.

A very simple stain which shows the nuclei well is a saturated solution of Potassium permanganate, applied to a dried film and gently heated for two minutes. Wash in water. The nuclei are stained deep brown.

The cells are generally oval in outline, though the shape varies somewhat in different species. Each cell is bounded by a thin cell wall and the interior consists of a granular vacuolated cytoplasm and a relatively large and somewhat vacuolate nucleus which will be deeply stained.

Many of the cells will be seen to have **buds** emerging from their sides, which represent the characteristic vegetative propagation of the organism. Each bud has its own nucleus, for nuclear division precedes the formation of buds. Each bud increases in size until it is as large as the parent cell. Either then or previously it separates from the parent cell and becomes an independent organism, itself beginning to form buds. Sometimes the cells fail to separate and short chains or groups may be produced. Under normal conditions only one bud at a time is produced by any one yeast cell. The rate of multiplication by this method is very rapid under favourable conditions. Stain another drop of material with Iodine. The vacuoles take a deep brown colour, due to the presence of **glycogen,** which takes the place of true starch as the chief reserve substance.

Spore Formation.—Prepare the following solution: 45 gm. of Beet root; 15 gm. Beet leaves infused in 150 c.c. of boiling water. Autoclave the solution, after straining through cheese cloth, for twenty minutes at

2 atmospheres pressure. To the solution add some yeast. In this solution spores may take some time to develop, but all sporulating strains react eventually. Spread some of the sporulating yeast on a slide and allow to dry up. Place the slide in absolute Alcohol for two minutes and then wash in Chloroform for two minutes to remove fats. Stain for ten minutes in Carbol Fuchsine. Wash and decolorize in 1 per cent. Sulphuric acid for a few seconds. Wash and counterstain in 1 per cent. Soluble Blue. The vegetative cells stain blue and the spores red.

The spores are produced normally in groups of four in each vegetative cell, the nucleus of which divides first into four, during which meiosis occurs. Around each nucleus a portion of the vegetative cytoplasm aggregates, and in the furrows between the portions, wall material is laid down. Since the parent yeast-cell is small it is necessary to examine the slide with the highest available power of the microscope. It may frequently be observed that less than four spores are visible at any one focus in many of the sporulating cells (Fig. 14).

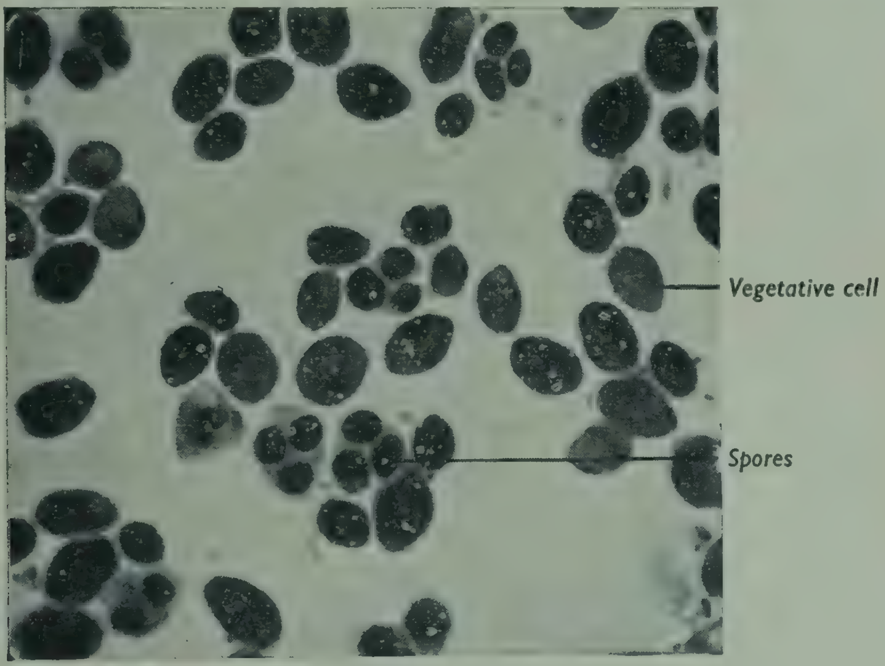

FIG. 14.—*Saccharomyces cerevisiae.* Sexual reproduction.

In the allied genus *Zygosaccharomyces* it should be noted that a conjugation of parent cells is necessary before spore formation can occur, but this is not true of *Saccharomyces*.

6. **Claviceps.** *The Sclerotium.*—The common species is *Claviceps purpurea*, which is the cause of the disease known as Ergot of Rye. The ascospores of the fungus germinate on the flowers of the Rye plant and give rise to a mycelium which at first spreads in the tissues of the ovary wall, later filling the whole of the ovary cavity. This structure

is the **sclerotium.** It is difficult to find early stages, and the preparation of the material calls for a microtome section.

In a well-prepared slide it will be seen that the mycelium spreads over the ovary forming a closely packed layer of short **conidiophores** from which minute oval **conidiospores** are abstricted in chains. These conidiospores can infect the flower of a new host.

At the same time that the conidiospores are maturing, **honey dew** is excreted which attracts pollinating insects. These become covered with the conidiospores which are thus distributed by the insects.

Examine an older sclerotium with a hand lens and note that as it matures it becomes larger and firmer in texture and brown or purple-black in colour. These sclerotia hang down out of the flowers as curved black structures about an inch in length. When the Rye plant matures they fall to the ground and remain there until the following spring.

In the spring the sclerotia germinate and several stalked **stromata** grow out from each of them. Each stroma is reddish-yellow and has a violet stalk.

Structure of the Stroma.—Cut a median vertical section through the stroma, stain in Ehrlich's Haematoxylin and make a permanent preparation. The cutting of the object by hand is difficult, and for detailed observation a microtome section is desirable.

The stroma has a **stalk** and a **perithecial head** in which numerous perithecia are embedded. These perithecia project above the surface of the head as small processes which are the outer ends of the **ostioles.**

The stalk is composed of fine, longitudinally running, septate hyphae which are more closely packed together towards the margin. The head is made up of a more delicate tissue of anastomosing hyphae, which radiate out from the top of the stalk and terminate in a layer of the closely packed ends of hyphae.

In the peripheral tissue the **perithecia** are developed as oval cavities, each opening to the exterior by an **ostiole.** The wall of the perithecium is lined by a more deeply staining tissue, and from the base of the internal cavity there grow up the **asci,** each of which is very slender. Towards the apex of the perithecium hyphae grow in from the margin and form a delicate system of threads partly blocking the ostiole. Each ascus produces a number of very fine thread-like **ascospores** which are difficult to differentiate from one another.

7. ***Hypoxylon coccineum.*** *External morphology.*—This Fungus is found on the bark of decaying branches of various kinds of angiospermic trees, but it is particularly common on Beech.

Each fruiting body is roughly hemispherical and about a centimetre in diameter. It is reddish brown in colour and is sometimes bright red. Older stromata lose their bright colour and become brown or almost black.

The fruiting bodies are often produced together in large numbers and there is a tendency for them to become confluent.

Sexual Reproduction.—Cut a vertical section through a mature stroma, stain in Ehrlich's Haematoxylin and mount in Canada Balsam.

In the section it is possible to recognize several zones. In the centre is an area in which the hyphae are loosely arranged and in which some cells of the host appear to be mixed with the fungal hyphae. Outside this is a dome-shaped mass of closely packed hyphae which lie parallel to one another. The cells are thick walled, apparently devoid of contents, and show a marked interlocking of adjacent files of cells, which adds to the rigidity of the structure. The superficial zone consists of radially running hyphae, whose walls stain deeply and whose cells contain some cytoplasm and show the same interlocking structure. Towards the margin of this zone lie the **perithecia,** each of which is rather rectangular in section. The perithecia are closely packed together and their adjacent walls are very dense and show little cellular structure. The perithecia contain **asci** and **paraphyses,** which arise mainly from the base of the perithecial wall. Each ascus contains eight oval **ascospores** which are purple-brown in colour. The paraphyses are very delicate, unbranched, translucent hyphae. The perithecia open by a slightly raised **ostiole** which is fringed on the inside by delicate hyphae. A rather delicate superficial layer covers the fruiting body through which the tops of the ostioles just project.

D. BASIDIOMYCETES

1. ***Puccinia*** (Wheat Rust). *Morphology of the Parasite on the Barberry.*—Examine a leaf of the common Barberry, *Berberis vulgaris*, infected by the Wheat Rust, *Puccinia graminis.* The **aecidial cups** occur on the lower side of the leaf, and may be either scattered or grouped together. Each cup is yellow in colour and has a raised margin around it. With a hand lens it is possible to see here and there tiny ostioles of the **pycnidia** on the upper surface of the leaf. Make a transverse section through a leaf, cutting both an aecidial cup and one or more pycnidia.

The **aecidium** is partly sunken in the tissues of the leaf, which become much modified as a result of the infection (Fig. 15). The wall of the aecidium consists of very fine hyphae with rather thick walls, which form a membrane which is slightly bent outwards at the upper edge. Inside the cup at the base are short parallel hyphae. Each of these cuts off alternately a large and a small cell at its upper end. Towards the top of the cup these large cells become differentiated as **aecidiospores,** while the smaller cells decrease in size and function as structures which help to separate the aecidiospores from one another.

The pycnidia are smaller than the aecidia and more flask-shaped, with a narrow, raised opening. They are filled with fine paraphyses, which are irregularly branched, with minute **pycnospores** interspersed among them. Almost the whole of the pycnidium is filled with these structures.

Uredosorus on Wheat.—The **uredosori** occur in long lines on the surface of the Wheat leaf. They are first formed below the surface of the epidermis, and at maturity break through the epidermal tissue to expose their spores. Cut a transverse section through the leaf and examine under the high power.

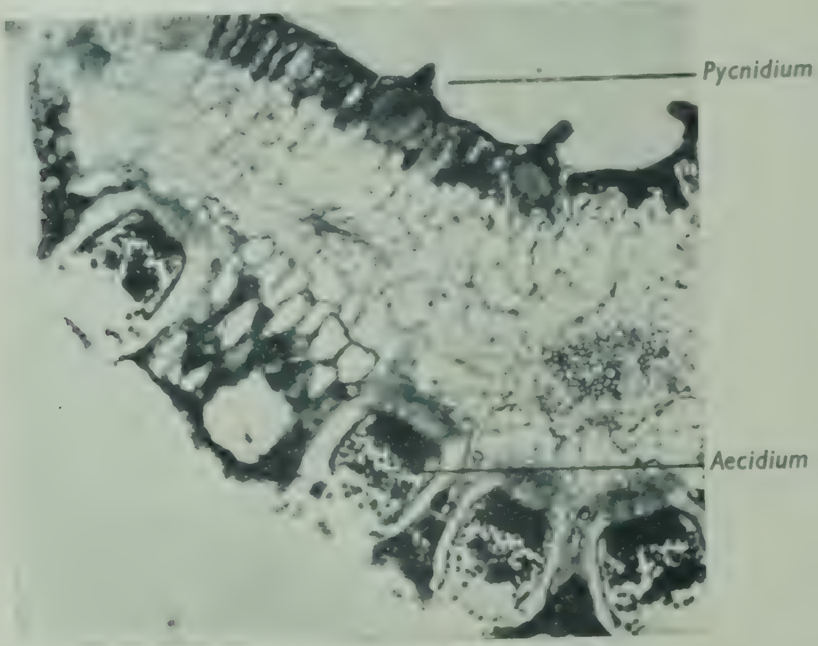

FIG. 15.—*Puccinia graminis.* Aecidia and pycnidia on Barberry.

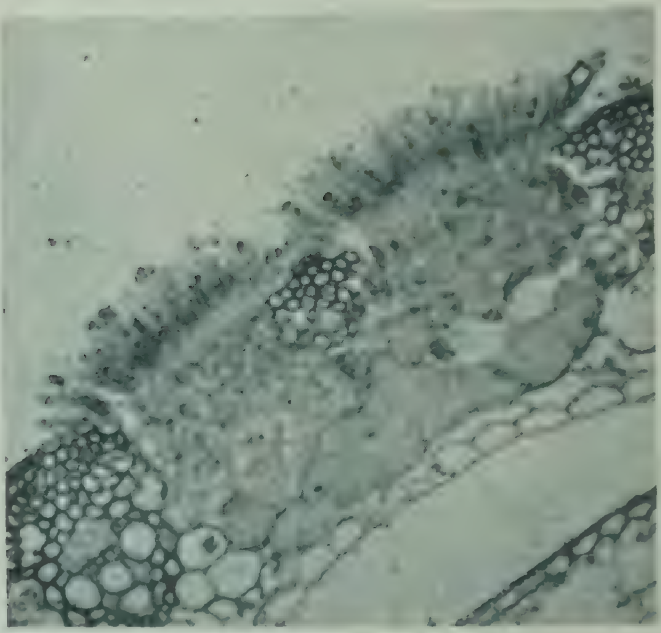

FIG. 16.—*Puccinia graminis.* Uredosorus on Wheat.

The uredosorus consists of groups of hyphae which grow out from the tissues of the leaf, forming a mass in which can be distinguished relatively thick hyphae which each bear one **uredospore,** and the fine **paraphyses** (Fig. 16). Each uredospore is binucleate and has a thin, bright yellow wall. The spore is oval in shape, generally slightly wider at the apical end. It is usually difficult to make out the details of the mycelium as it passes between the cells of the host plant, but it will be noticed that the thick-walled sclerenchyma surrounding the vascular bundles is not affected, only the thin-walled mesophyll cells between them becoming disorganized.

The uredospores on liberation germinate directly on the Wheat and produce a mycelium from which more uredospores arise during the summer months. Later in the autumn the same mycelium will produce teleutospores (see below).

> *Teleutosorus on Wheat.*—The sori of the teleutospores occur in the same position as the uredosori, but may be distinguished in the field by their darker colour. Cut a transverse section of the leaf through a teleutosorus and examine under the high power.

In a transverse section of an infected leaf the **teleutosorus** appears very similar in general morphology to the uredosorus. The **teleutospores** themselves are quite unlike the uredospores (Fig. 17). They are bicellular, each cell being more or less triangular in outline. They have dark brown walls which are relatively thick, but a thin **germination pore** can be seen in each cell, one at the apex and one just below the partition wall. In the mature spore the two nuclei, which are present in each of the young cells, fuse.

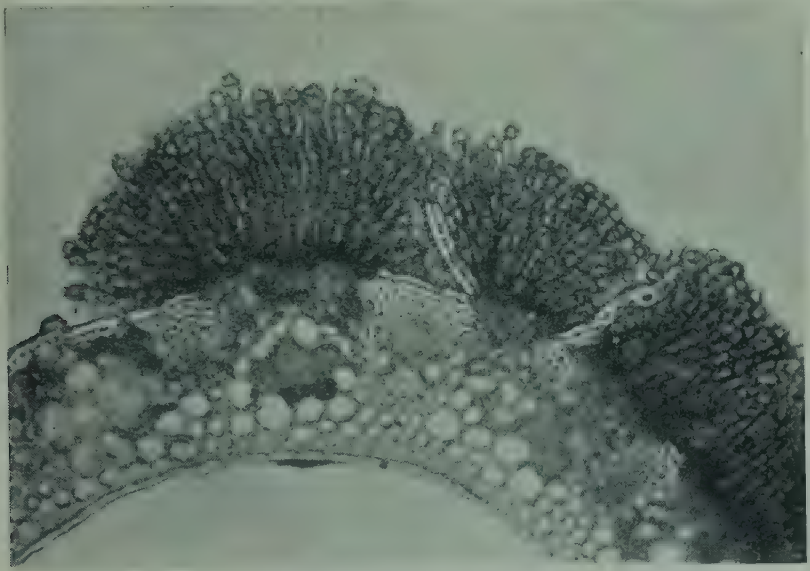

FIG. 17.—*Puccinia graminis.* Teleutosorus on Wheat.

Occasionally, in favourable material, some of the teleutospores will be found to have germinated. From the pore of each of the two cells a short hypha grows out, which becomes transversely septate into four cells. From the upper end of each cell a **sterigma** is formed, from the distal end of which a single oval, thin-walled **basidiospore** is produced. It is these basidiospores which ultimately reinfect the leaves of the Barberry the following year.

2. **Polyporus betulinus.** *External Structure.*—The fruiting bodies are produced on the stems and main branches of the Common Birch.

Each fruiting body when mature is semicircular in outline with a wavy, slightly downwardly curved margin. The sporophore is mouse grey above but pure white below and is attached to the tree by a relatively small, very short stalk, or it may be completely sessile. The upper surface is smooth and leathery but the lower surface is soft and covered with minute pores which are the ends of the hymenial tubes.

Vertical Section of the Fruiting Body.—Cut a vertical section through a mature fruiting body, stain in Cotton Blue and mount in Lactophenol.

The fruiting body is divisible into several distinct layers. On the upper surface is a narrow zone of thick-walled hyphae, which run parallel to the surface of the sporophore. Below this is a wide zone made up of fine anastomosing hyphae, which appears to form a delicate plectenchyma.

Below this comes the wide zone of **hymenial tubes.** The hyphae of the sporophore run down longitudinally between the tubes, giving off, laterally, short hyphae from which the lining layer of the tubes is formed. This layer forms the **hymenium** and stains deeply. The **basidia** are short and rounded. Each bears four oval **basidiospores** on very short **sterigmata.**

At the lower surface the longitudinally running hyphae turn at right angles and in a young fruiting body will be seen to form a thin covering over the ends of the tubes. As the hymenium matures the tips of these hyphae disorganize, leaving **pores** whereby the tubes are placed in communication with the exterior and the basidiospores, as they are shed, are enabled to fall down the tubes and reach the exterior.

3. **Psalliota.** *External Morphology.*—Cut in half longitudinally a mature fruiting body of *Psalliota campestris*, the Common Mushroom, and examine under a hand lens.

The body consists of a stalk or **stipe** which, at its lower end, is attached to the underground mycelium and at its upper end bears the convex cap or **pileus.** Attached to the lower surface of the pileus are the gills or **lamellae,** which radiate outwards from the stipe to the margin of the pileus. Each gill is a thin layer of tissue, bearing **basidia** on which **basidiospores** are developed, which are purple in colour when ripe. Midway up the stipe is a ring or **annulus** which marks the position where the **partial veil,** which at an early stage encloses the gills, was attached to the stipe.

Amanita phalloides (the Vernal Toadstool) and *A. muscaria* (the Fly Agaric).—For comparison these two species may be examined and the differences from the Mushroom noted. In the Vernal Toadstool the same structures occur as in the Mushroom, but at the base of the stipe a cup-shaped structure occurs called the **volva,** which is a remnant of the **universal veil,** which is split by the elongation of the stipe during development of the fruiting body. A similar structure is found in the Fly Agaric, where, in addition, remains of the veil may be found as yellow spicules on the top of the cap.

These two species are among the most poisonous British Fungi, and can be easily distinguished from the Mushroom by the presence of a volva and by the white spores and gills in contrast to the purple spores of the Mushroom, which give a pink colour to the gills of young specimens and ultimately a black colour when mature.

Section through a Young Sporophore.—Cut a longitudinal section through a young fruiting body in the " button " stage.

The whole structure is oval in outline and is composed of tightly packed hyphae, each of which is very fine and is provided with cross walls. About one-third the distance from the upper end two small flattened cavities lying side by side may be seen (Fig. 18). These are the gill cavities, and between them is the rudimentary stipe. It will be noticed that the cells of the hyphae in this region are very short, due to repeated cell division without elongation. The upper surface of the cavities stains more intensely, and this is the tissue which will later give rise to the basidial layer. These two cavities are really

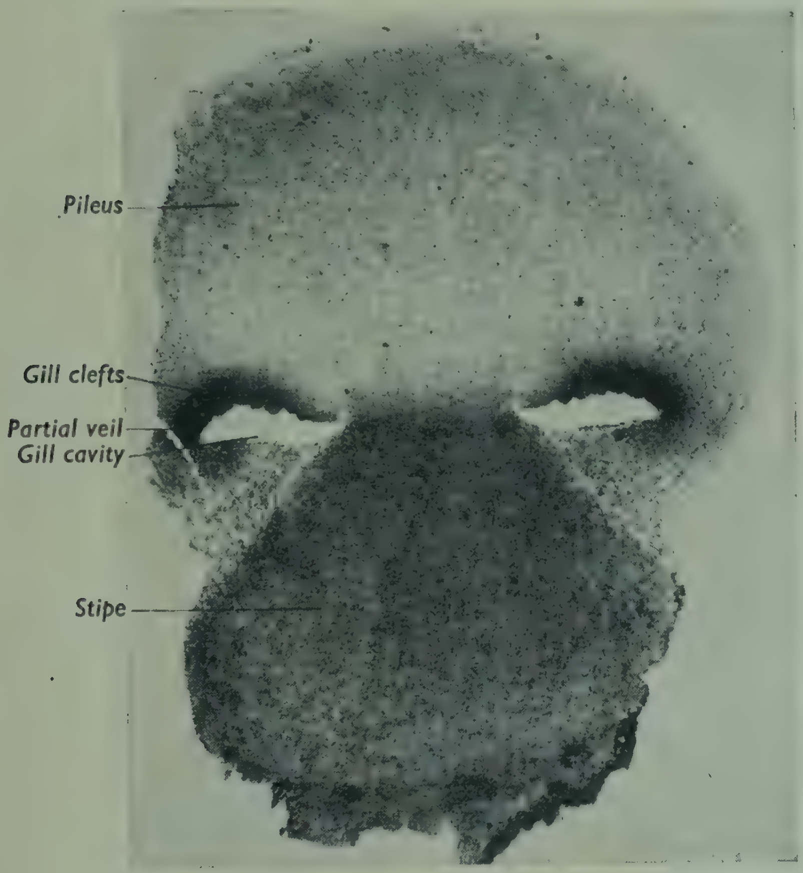

FIG. 18.—*Psalliota campestris.* Vertical section through young sporophore.

parts of a ring-shaped channel which is formed around the fruiting body at this stage. The outer layers of the fruiting body are composed of more closely packed tissue and include the partial veil. The basal part of the sporophore passes directly into the tissues of the supporting mycelium from which it originated.

> *Section through the Mature Pileus and Gills.* Cut a vertical section through the cap, including several gills. Stain in Cotton Blue and mount in Lactophenol.

The longitudinal section through the gills shows them to consist of a hyphal tissue bearing on its outer surface the **hymenial layer.** This can be easily recognized by the more intense staining which it assumes. The layer consists of hyphae which run transversely out to the surface and form club-shaped **basidia** interspersed with more slender **paraphyses.** Each basidium bears a pair of slender outgrowths or **sterigmata** at its outer end, and attached to each is a large oval **basidiospore.** In the cultivated mushroom there are only two basidiospores and two slender sterigmata borne on each basidium, whereas in the wild species, as in other toadstools, the characteristic number is four.

The central tissues of the gill consist of loosely packed longitudinal hyphae which form what is termed the **trama.**

The basidiospores are forcibly ejected from the hymenial layer and fall freely between the gills, provided that these are truly vertical. If, however, this were not so the spores would fall on the surface of an adjacent gill. The Mushroom fruiting body, therefore, is sensitive to gravity, and if held so that the gills are not vertical the stipe bends over rapidly till the gills return to the vertical position. This can be easily demonstrated by clamping the base of the stipe with a retort clamp in a horizontal position and leaving it for a short while in a humid atmosphere, when it will be seen to bend up at right angles, thus bringing the gills into a vertical position. This change is accomplished in quite a short time, but if watched with care it will be seen that the final position is arrived at by a kind of trial and error, since the fruiting body first passes the vertical and then fluctuates back and forth till the true vertical is arrived at.

> 4. ***Cyathus striatus.*** *External Morphology.*—The fruiting bodies are produced on the ground among dead wood and leaves in woods, during the autumn.

The fruiting body is like an inverted bell and is reddish brown on the outside and lead coloured within. The exterior is distinctly hairy and is sometimes longitudinally ribbed. These characteristics, however, are not shown by all species. In *C. striatus* the fruiting body is about 1·5 cm. high.

In the young condition the top of the fruiting body is covered by a flat, thin membrane, the **epiphragm,** but when mature this epiphragm is disorganized to expose a number of oval, whitish bodies, the **peridiola,** each of which is attached to the wall of the fruiting body with a fine extensible thread, the **funiculus.**

Longitudinal Section of the Fruiting Body.—Cut a longitudinal median section through a mature fruiting body, preferably with a microtome. Stain in Ehrlich's Haematoxylin and mount in Canada Balsam.

The wall is composed of several layers and is made up of fine hyphae which, externally, are somewhat loose and free. This is followed by a zone in which the hyphae run mainly longitudinally forming an interwoven tissue which is brown in colour. Inside this is a zone of true plectenchyma, the component cells of which have very delicate walls. This is followed by a narrow zone of longitudinally running hyphae, similar in construction to the outer zone. On the inside is a looser tissue of brown hyphae bounding the inside of the fruiting body. The outer zone is the **exoperidium,** the inner zone the **endoperidium.**

Attached to the endoperidium are the **peridiola,** each of which is attached by the **funiculus.** This latter body is attached to the wall by its basal end and contains a mass of very fine, spirally twisted hyphae which are attached to the peridiolum by the nipple-like upper end. The **peridiolum** is oval in section and consists of a thick outer wall, inside which is a rather amorphous tissue containing a considerable amount of gelatinous material. In a mature peridiolum the centre is filled up with a mass of small, oval **basidiospores.** Internally around the margin of this central cavity may be seen, here and there, **basidia** in process of development.

5. ***Phallus impudicus.*** The fruiting body can be readily recognized by the offensive smell which it emits. In the young condition it consists of a spherical, white, gelatinous mass about the size of a hen's egg. Cut a young fruiting body in half vertically and examine with a hand lens. Alternatively, embed a young fruiting body in paraffin wax and cut serial sections with a microtome. Select a median section for study.

On the outside is the **volva,** which is thin and delicate. It encloses the whole fruiting body in the young stage, but will be ruptured at maturity and will then appear as a cup around the base of the stalk. In the centre lies the **stipe** or stalk of the fruiting body which, in section, will appear to be compressed and concertina-like, in the egg stage. It is the sudden rapid expansion of the stalk which ruptures the volva. Borne on the top of the stipe is the **pileus,** a dome-shaped mass of tissue, rather delicate in construction, which bears, on its convoluted outer and upper surface, the **gleba.** From the gleba the **basidiospores** are formed and will already be recognizable as a dark, greenish-grey mass. If the fruiting body is very young, the gleba may still be covered by a thin delicate membrane, the **endoperidium.** Between the endoperidium and the volva the layers will have largely broken down to form a gelatinous material, unless the fruiting body is extremely young.

Section 3. Bacteria

1. **Bacillus.** *Cultivation, Staining and Examination.*—*Bacillus subtilis* and its closely allied species are useful bacterial types for study, as, on account of the resistance of their spores to boiling, they are easily isolated.

Take a handful of dry hay or straw, place it in a flask and pour water over it. Boil for half an hour, plug the neck of the flask with cotton wool and leave it in a warm place for a day or two. The water will become cloudy with the growth of *Bacillus subtilis.*

A small drop of this culture may be transferred to Agar, with a platinum wire sterilized in the Bunsen flame, when it will develop into a thick surface growth which may be preserved and occasionally subcultured, if desired, as a permanent stock.

Place a drop on a very clean microscope slide and dry it with the aid of gentle heat. When dry the slide is rapidly passed through the Bunsen flame three times, *bacteria uppermost*, to warm the slide enough to " fix " the organisms to the glass. It should not be too hot to bear comfortably on the back of the hand.

The dried film may be stained with a simple stain such as 1 per cent. Methylene Blue, applied for two to three minutes, and then washed off with a gentle stream of water. The **Gram stain** may also be used, and as this is the standard bacteriological stain, it is well to learn to use it.

Stain the film for one minute with 1 per cent. Crystal Violet in water. Drain off the surplus stain without washing and add a few drops of a solution of about 1 per cent. Iodine in 2 per cent. aqueous Potassium iodide. Leave for one minute. Wash off with 95 per cent. Alcohol and continue washing for about two minutes, or as long as any colour comes out. Wash in water and counterstain for thirty seconds in 1 per cent. aqueous Neutral Red. Wash and dry. Bacteria are classed as **Gram positive** or **negative** according to whether the violet stain is held or washed out in the spirit. *Bacillus subtilis* is Gram positive. Mount the film in Balsam or, if for temporary use only, in thick Cedar Oil or Ethyl phthalate. For use with an oil-immersion lens, it need not be mounted at all.

Examine the film with the highest magnification available. It should be first found under the lower power, and then the high power turned on. The cells appear as short rods with rounded ends, and of various lengths, according to the state of division the cells are in. Some of the cells show **spores** (Fig. 19). These appear as hollow ovals in the body of the bacterium, with the contents unstained. The spore first forms as a dense granule of deeply stained matter in the bacterial protoplasm. As it develops it appears to become hollow, and when it is mature the cell round it disintegrates and the oval-shaped or spherical spore is left lying loose. It has remarkably dense and impermeable walls and a high refractive index, which causes it to shine out brightly in an unstained preparation.

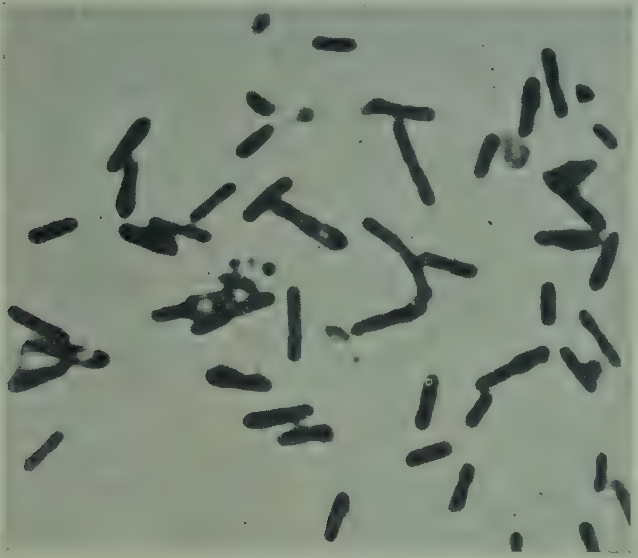

FIG. 19.—*Bacillus sporogenes.* Individuals with endospores.

Stages in spore germination may also be seen, the spore opening laterally and a new short bacillus growing out from its contents.

2. **Staphylococcus.** *Description of Organism.*—The cocci are minute spherical bacteria, usually occurring in groups and seldom singly. *Staphylococcus* is the name given to those which are aggregated into irregular clusters like grapes. Prepare a tube or plate of sterile Agar. Rub the end of a sterilized platinum wire under the finger-nail and rub it in a drop of boiled water placed on a flamed slide. Take a small drop of this water on the end of the wire and pass it gently over the Agar surface in a series of parallel streaks. Alternatively rub the wire against the skin and then streak it directly on the Agar. This method is apt to produce too thick a growth.

Incubate at 37° C. for twelve to twenty-four hours, or for several days at room temperature. The Agar will show a large number of dull yellowish colonies and probably a smaller number of white colonies. These are *Staphylococcus aureus* and *S. albus* respectively.

Make and stain films as described for *B. subtilis*, using the Gram stain.

Under the microscope thick aggregations of Gram positive (purple) cocci will be seen clustered together like bunches of grapes. Each coccus is about 1 micron in diameter and too small therefore for any internal detail to be visible.

Staphylococcus aureus is almost universal on the human skin. It is harmless to the digestive organs, but causes most of the small inflammations of the skin when it gains access to a cut or scratch.

Section 4. Charales

> 1. **Chara**. *External Morphology.*—There are various species of *Chara* which differ in detail, but the main characteristics are common to all. They can be easily cultivated in glass jars in the laboratory.

The plants are submerged aquatics, from 1 to 2 ft. in height. The stems are long and slender, with very long **internodes,** consisting of a single coenocyte surrounded by a **cortex** of very long, delicate cells which can be seen as striations on the surface of the internode, following a slightly spiral course.

At each **node** there arises a whorl of six or eight **branches** of limited growth, about 1 to 2 cm. long. These also are divided into nodes and internodes. At the branch nodes, in fertile specimens, are borne the orange-coloured **oogonia** and the whitish **antheridia,** both large structures visible to the naked eye. They are frequently in pairs at the same node, the oogonium above and the antheridium below, and they are surrounded by a small group of secondary branches, one or two cells long.

Growth is apical and there is a conspicuous **apical bud** of branches at the top of each main stem.

The whole plant is often encrusted with a deposit of Calcium carbonate, the wall of the oogonium in particular becoming almost mineralized and turning black when mature.

> *Sexual Reproduction : Oogonium.*—Pick off a few young oogonia, dehydrate them for a few minutes in absolute Alcohol and mount in Clove Oil. Alternatively, mount them in Lactophenol.

The oogonium is never a very transparent object and in the older stages it becomes quite opaque. It is not however difficult to see the **enveloping cells,** which are spirally wound round the **oosphere,** which contains abundant droplets of fatty oil. At the top the enveloping cells are capped by a group of small cells, called the **coronula,** which forms a neck to the oogonium. At the base may also be seen the **basal** or **turning cell** on which the oogonium rotates during its development.

> *Sexual Reproduction : Antheridium.*—Pick off a mature antheridium. Tease out in a drop of Lactophenol with Cotton Blue and mount in Lactophenol.

The antheridium is covered by a series of plates called **shields,** from the centre of each of which arises a columnar cell, the **manubrium.**

These are directed to the centre of the whole antheridium and at the inner end of each is a small group of cells, the **capitulum.** From each capitulum there arises a bundle of long **spermatogenous filaments,** divided into small, discoid cells, which produce the **antherozoids.** These can usually be seen curled up in the cells and some may be liberated during the mounting of the preparation. Each has a pair of **flagella.**

Section 5. Hepaticae

1. **Pellia.** *External Morphology.*—There are three species of *Pellia* in Great Britain, all very similar, of which *Pellia epiphylla* is the commonest.

The plant consists of a flat green **thallus** which is dichotomously branched. The margin of the thallus is slightly frilled and down its centre is a well-marked, purple-coloured **midrib.** Numerous short **rhizoids** arise from the lower surface in the region of the midrib, by means of which the plant is attached to the soil on which it grows. The segments of the thallus are rarely more than 2 in. in length, due to the fact that the older parts decay, leaving the younger branches to develop independently.

On the mature female plant may be found the **sporogonia** which arise from the upper surface in the cleft between two apical segments. Each consists of a white stalk 1 to 2 in. in length and terminates in a small spherical black **sporangium.**

Generally the plant grows in large masses, comprising many separate thalli which overlap one another. *Pellia* grows in damp situations, on the soil in shady hedgerows, on the banks of streams and similar places.

Structure and Sexual Reproduction: Antheridium.—Transverse section of the thallus about 1 cm. from the apex, mounted in Glycerine.

Examination of the thallus with a hand lens will show a number of small dark dots in the region of the midrib. These are the antheridia, and it is through a group of these that the section should be cut.

The vegetative part of the thallus is entirely parenchymatous in structure. The wings are only two to three cells thick, but it is enlarged at the midrib into a tissue about a dozen cells deep. Irregular bands of cuticularized thickening are to be seen on the walls of some parenchyma cells, especially in the midrib, but they are not always visible and may require to be looked for with the high power. In one British species, *P. fabbroniana*, they are missing. When present they stain well with Safranin. From the lower surface of the midrib grow out the rhizoids, simple unicellular tubes which act as anchoring and absorptive organs like the roots of higher plants.

Examine the cells in a thin part of the thallus with the high power, and note that the numerous chloroplasts are arranged in close order on the upper surface of the cells, where they are most exposed to light. This is no doubt a result of the heavy shade in the places where *Pellia* mostly grows. This position of the plastids has been called **epistrophe,** in contrast to the more usual position on the side walls, which is known as **apostrophe.**

The interior cells of the thallus contain few chloroplasts, but have numerous grains of storage **starch** which colour purplish-blue with dilute Iodine.

The lower cells, especially near the midrib, frequently, though not invariably, contain a mycorrhizal Fungus which may be distinguished as fine threads forming an irregular network inside the cells.

The **antheridia** are spherical and are produced just below the upper surface, arising from a cell which is in origin superficial. Each consists of a single-layered wall enclosing a mass of small cubical cells each forming one **antherozoid.** If an antheridium in process of dehiscence is found, the antherozoids may be seen as minute worm-like bodies escaping from the top of the antheridium. Each has two long, backwardly directed **flagella.**

The antheridium wall separates from the thallus cells around it, so that it lies in a spherical cavity, attached only by one or two cells at the base which form a sort of plinth or foot. Even these eventually are destroyed in the general solution of the antheridial wall at maturity. Meanwhile the superficial cells immediately above the antheridium grow upwards to form a rosette-like crown. The rosette enlarges and opens, leaving the top of the antheridium exposed. The surrounding tissue expands, and the whole structure, now a mass of ripened antherozoids, is eventually raised above the surface surrounded by the ring of rosette cells. At this stage the antherozoids are released by the solution of the mucilage in which they are embedded, and only the empty cavity remains.

> *Sexual Reproduction : Archegonium.*—The archegonia must be looked for close to the apex of the frond. Look for a little pocket formed by a minute flap on the surface of the thallus, close behind the growing point. The archegonia grow in a group inside this pocket, and are best seen in longitudinal vertical section. Choose a pocket in the youngest condition you can find. The larger ones are probably those from which sporogonia have already grown and dropped off.

A successful section will show that the flap, known as the **involucre,** arises from a thickened portion of the thallus which bears the **archegonia,** which are thus to be found in large numbers underneath the involucre.

The mature archegonia have a rather thick **neck,** twelve to fifteen cells in length, which widens gradually downwards into the **venter,** without any clear division between them. They grow in three superimposed layers on the almost vertical face of the thickened portion of the thallus with the necks towards the apex of the frond, *i.e.*, towards the opening of the involucral pocket.

The cushion of thickened tissue on which they stand is small-celled and densely packed with starch reserves. Later, when an embryo is developed, its foot burrows down into this tissue and withdraws the reserve food from it.

> *Longitudinal Section of Young Sporophyte.*—The sporophyte grows out from under the involucre and can be seen as a little globular swelling even before it emerges. Look for one of the youngest recognizable sporophytes, still covered by the fringed involucre, and cut a longitudinal section. Sections will need to be fairly thick to keep the parts together, but this does not matter as the tissues are fairly translucent. Mount in Glycerine.

The involucral pocket will appear to have deepened considerably from the pre-fertilization stage, as it now holds the half-grown sporogonium, both **theca** (capsule) and **seta.** This is due to two causes—the lengthening of the involucre itself, and the backward growth of the foot of the seta into the starch-loaded tissue of the thallus.

The position of the original archegonium is marked by the membranous remains of the **calyptra,** which is the enlarged wall of the fertilized archegonium. This is burst through by the growing sporophyte, and eventually forms a loose collar round the base of the seta, inside the involucre. The seta has a pointed lower end formed by a conical **foot,** not unlike the root-cap of an Angiosperm, by which contact is maintained with the gametophyte tissues and food solution absorbed. The seta itself contains abundant starch in the young stages and the cells are short and compact, but when the spores are mature these cells very rapidly elongate, the starch is consumed and the seta grows upwards carrying the capsule to a height of 2 to 3 cm. above the thallus.

In the earliest stages the capsule is occupied by a solid mass of cells, the **archesporium.** At a later stage these cells separate from one another and become **spore mother cells.** They then become deeply four-lobed, with the lobes arranged pyramidally, and when meiosis has occurred each lobe receives one haploid nucleus and is separated from the others to become a **spore.**

If the capsule shows only mature spores refer to the next section for the description.

> *Longitudinal Section of Mature Sporogonium.*—A longitudinal section of a nearly mature spore capsule should be cut fairly thick so as to retain some of the spores in position.

The spores are oval bodies with granular yellowish walls. They usually show **precocious germination,** that is, they begin to divide before release and develop into several-celled individuals. The spores of *Pellia* are peculiar in containing chlorophyll. They have no resting period and continue growth as soon as they are shed.

Among the spores are the **elaters,** narrow, pointed cells, often twisted, with no contents and with from two to four **spiral bands** on their walls. They are arranged among the spores, at first roughly in lines radiating from a solid mass of elaters at the base of the capsule, but this is soon obscured by the twisting of the elaters, due to the hygroscopic quality of the spiral bands.

In a thin section it should be possible to see that the wall of the capsule is formed of two layers of cells. The outer cells are large and cubical in shape, with a thick external cuticle and side walls which are thickened with the same yellow material that occurs in the elaters. The layer strongly suggests the annulus of a fern sporangium. The inner layer consists of narrow cells with a thick yellow reticulum on the walls. This reticulum is sometimes more or less spiral, and the cells seem to be closely related in form to the elaters. The capsule wall breaks open along four lines, from above downwards, the wall folding outwards in four valves and exposing the spore mass, which is rapidly broken up by the hygroscopic movement of the elaters. All that remains is the little basal tuft of attached elaters, sticking up like stamens in a lilliputian flower.

2. **Marchantia.** *External Morphology.*—The thallus of *Marchantia polymorpha*, the only British species, consists of a flat, branched structure which may be easily distinguished from other species by the gemma cups which are developed on the upper surface.

The **gemma cups** are circular in outline, formed by an upgrowth of the margin of the thallus, and are somewhat urn-shaped. Inside are numerous bright-green **gemmae.** The thallus itself is tough and leathery, branched and deep green in colour. Along the centre line is a **midrib** which projects slightly on the lower surface. From it arise numerous **rhizoids** and also somewhat incurved scales or **amphigastria.** These latter are often purple in colour.

Through a hand lens may be seen on the upper surface small dots, which are the **pores** by which communication is maintained with the chambers below in the tissue of the thallus. It is by means of these that gaseous interchange takes place. Each pore lies in the centre of a diamond-shaped area which shows the extent of one of the sub-epidermal assimilatory cavities.

The sexes are separate and the reproductive organs are produced on umbrella-shaped structures, termed respectively **antheridiophores** and **archegoniophores,** bearing the male and female sex organs upon them. Since the sporogonium develops on the archegoniophore, no seta similar to that found in *Pellia* is developed in this species.

Transverse Section of Thallus.—Cut a transverse section through the thallus. Stain with Cotton Blue and mount in Lactophenol.

An **epidermis** of a single-celled layer forms the upper surface. Below this lies a stratum of flat chambers separated from each other by vertical walls of cells, which show externally as diamond-shaped markings on the surface of the thallus. Each of these large **assimilatory chambers** opens to the outside by a centrally placed **pore.** Each pore is surrounded by rings of cells in tiers, of which there are four or five, standing one on top of another so as to form a barrel-shaped structure open at both ends. Each ring consists of four cells, and those of the lowest tier bulge inwards so that they partially close the opening, which is thus reduced to a cross-like slit. There seems reason to believe that these cells have some regulatory power over the opening between them, like the guard cells of a stoma.

The chambers contain short filaments of cells, rich in chloroplasts, which are in contact with the air and seem to be the chief photosynthetic tissue of the thallus.

Below the assimilatory chambers the tissue becomes parenchymatous, containing starch grains but few chloroplasts. There is little differentiation, but the thallus is thicker at the midrib, and there are mucilage storage cells here and there. The **lower epidermis** bears many unicellular **rhizoids,** some smooth-walled and some with **tubercles** projecting into the cell cavity, which are characteristic of the Marchantiaceae.

On both sides of the midrib there arise, in addition to the rhizoids, multicellular structures which are the **amphigastria** or ventral scales.

If a section has been cut through a **gemma cup** it will be seen that the upper superficial tissues of the thallus are carried up to form the walls of the gemma cup, and that the gemmae themselves develop from the lower parenchymatous tissue. In a section the gemmae appear of various sizes, each being made up of small parenchymatous cells rich in chloroplasts. There is a notch at each end of the oval-shaped body, and in each notch is a growing point, so that when the gemma starts to grow it does so in two directions at once. The tissues of the gemma are quite undifferentiated, and the future upper and lower surfaces are determined solely by external conditions at the time of germination.

Here and there in the thallus tissue may be seen cells with glassy, slightly yellowish contents. These are mucilage cells and apparently serve to retain water in the tissues in dry weather.

External Morphology of Antheridiophore and Archegoniophore.—The structure of the antheridiophore can be seen best with a hand lens.

It is borne on a long slender stalk which at the top forms an umbrella-shaped, flat or convex cap. This cap consists of a scalloped margin comprising normally eight lobes, which are broadest towards the centre. The whole cap is green in colour, due to the presence of chloroplasts in chlorophyllous tissue developed exactly like that in the thallus. In fact, the stalk and cap are really metamorphosed branches of the thallus.

The archegoniophore is similar in general appearance to the antheridiophore, and consists generally of nine narrow rays, between which are eight groups of archegonia borne on the under side of the receptacle. Like the antheridiophore it is borne on a stalk, generally longer than that of the antheridiophore. In colour and in vegetative structure it agrees with the antheridiophore.

Transverse Section through Stalk of Antheridiophore.—Cut a transverse section through the stalk of an antheridiophore. Stain in Cotton Blue and mount in Lactophenol.

The stalk in section looks like a very narrow branch of the thallus. On the dorsal side it has a layer of assimilatory chambers, with pores, and on the ventral side there are two canals, formed by the infolded edges of the stalk overlapping two ventral flanges of tissue, which correspond to amphigastria. Rhizoids which originate on the underside of the cap run downwards in these canals.

It has been suggested that since in Nature these canals will be filled with water as a result of capillarity, they may well provide a means whereby the antherozoids make their way down the antheridiophore and again swim up the archegoniophore, for that organ develops a stalk exactly like that on the antheridiophore. As the sexes are on separate plants, not necessarily growing close together, this suggestion is of dubious possibility. Another and more likely one is that the antherozoids are carried to the female plants by mites.

> *Longitudinal Section of Head of Antheridiophore.*—A longitudinal section through the head of an antheridiophore may be made by enclosing the material in a split piece of pith. Median sections are preferable, since in them the attachment of the stalk may be studied. Stain in Cotton Blue and mount in Lactophenol.

The tissues of the cap are similar to those of the vegetative thallus. On the outside is an epidermis, with pores for gaseous interchange, identical in structure with those on the vegetative thallus already described. The same chambers occur, bearing, from basal tissue, filaments of chlorophyllous cells. The lower tissues consist of parenchyma, and on the lower epidermis are borne scales and tuberculate rhizoids.

The **antheridia** occur in cavities in the upper surface and are arranged in radiating rows, the youngest towards the margin. Each antheridium is supported on a single short stalk cell and is oval in outline and lies freely in a cavity. The antheridium is a large multicellular body composed of many very small cells. There is a single-layered wall around the antheridium, all the internal tissue consisting of **antherozoid mother cells** destined each to produce a single biflagellate **antherozoid.**

> *Longitudinal Section of the Head of Archegoniophore.*—Cut a median longitudinal section through the top of an archegoniophore in the same way as the antheridiophore. Stain in Cotton Blue and mount in Lactophenol.

The structure of the vegetative tissues is similar to that of the antheridiophore. The archegonia occur in groups on the lower side, one group between each pair of rays (Fig. 20). The archegonia develop in succession from the centre of the umbrella outwards, and each series is enclosed by a veil-like covering with a frilled margin, which develops on either side of each row of archegonia and protects them during their early development. This is the **involucre,** corresponding to that in *Pellia.*

Each archegonium consists of a short stalk, a body or **venter** and a **neck.** Each is surrounded at its base by a small membranous cup, the **perianth,** which does not develop to any extent after fertilization. The wall of the archegonium consists of two layers of cells. In the centre of the body are two cells, the **oosphere** and the **ventral canal cell,** which are sister cells, though the oosphere alone is capable of being fertilized. Above these in the neck region there is a row of **neck canal cells.** Between the archegonia there arise numerous small scales which may function as paraphyses.

Longitudinal Section of Sporogonium.—Choose one of the older archegoniophores in which the young sporogonia can be distinguished as minute rounded swellings on the under side. Cut a median section, which should pass longitudinally through one or more sporogonia. Microtome sections are preferable, though excellent results may be had by hand.

The young sporophyte remains enclosed in the enlarged wall of the archegonium until it is several millimetres long. This covering is known as the **calyptra.** It is the third envelope around the embryo, the other two being the perianth and the involucre.

The distinction between seta and theca is established at a very early stage, the seta cells being larger and emptier than those filling the theca (**archesporium**). The seta ends in a **foot** of large densely filled cells of glandular appearance, which remain in union with the base of the archegonium. The seta never elongates, as in the upside-down position of development this would defeat its object. The theca soon becomes oval, and the archesporial cells elongate. Some divide transversely to form longitudinal rows of cells which become **spore mother cells.** Others lose their nuclei, elongate further and soon acquire spiral thickenings. They become **elaters.** The mother cells undergo meiotic division when the whole sporophyte is about 1 mm. long and form tetrads of small **spores.** At this stage the capsule wall is still soft and its cells contain curiously barred starch grains, but when the spores and elaters are ripe the wall cells develop thickened bars, like a pair of crossed horseshoes, which contract when dried and cause irregular tearing of the wall, liberating the spores.

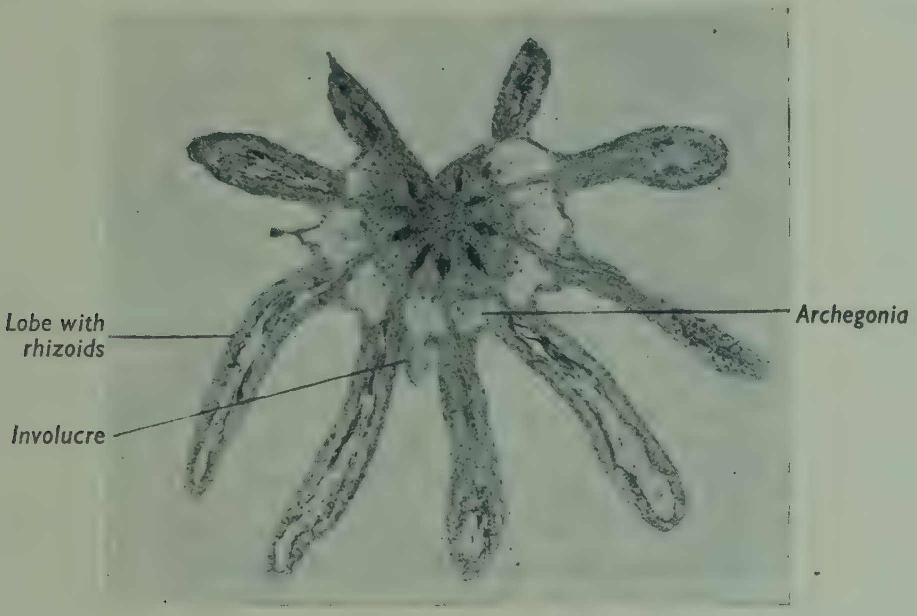

FIG. 20.—*Marchantia polymorpha.* Horizontal section through the archegoniophore.

3. ***Anthoceros laevis.*** *External Morphology.*—This Liverwort is by no means common, but it is found chiefly in the northern and western

parts of Britain, growing in moist hollows or on shady banks, often in abundance. Examine a portion with a hand lens.

The thallus is richly lobed, the lobes being often closely packed together. It is of a translucent green when fresh, and the lobes have crenate margins so that the plant has a frilled appearance. Smooth-walled **rhizoids** are formed on the lower surface. The position of the groups of **antheridia** is shown by raised points on the upper surface, usually of a yellowish colour. The **archegonia** are sunk in the tissue and they are scarcely discernible with a hand lens. The sexes occur on different plants.

The **sporogonia** are 1 to 2 in. long when mature and grow from the upper surface of the thallus in considerable numbers. They are delicate cylindrical structures and each is surrounded at the base by a **calyptra** sheath.

Structure of the Thallus.—Cut a transverse section of a young thallus lobe and mount in Glycerine.

The thallus has a surface layer of cells which are smaller than those of the rest of the thallus but not otherwise different. The thallus is entirely parenchymatous and contains numerous large **mucilage cavities,** except at the edges. Each cell has one large and irregularly shaped **chloroplast,** containing a conspicuous **pyrenoid,** and numerous small **starch grains.** Here and there the dense, spherical colonies of the endophytic Alga, *Nostoc*, may be seen. These inhabit special cavities in the tissue, surrounded by several concentric layers of flattened cells. Filaments of cells may also be seen penetrating among the algal trichomes in the cavity.

Sexual Reproduction : Antheridium.—Cut a transverse section of a thallus lobe on which antheridial pits can be seen. Mount in Glycerine.

The **antheridia** are formed endogenously in small groups and are covered over by a thin roof of thallus cells, which breaks open when they are mature. Each antheridium is borne on a short multicellular stalk. It has a single-layered wall and is somewhat bean-shaped. Three to five antheridia are formed in each group.

Sexual Reproduction : Archegonia.—As the archegonia are formed singly their presence can only be recognized by the minute projection of the end of the neck from the upper surface of the thallus. Cut a number of sections across a mature thallus lobe. Mount in Glycerine and look for archegonia. If no antheridia are visible, archegonia may be expected.

The **neck,** four to six cells long, is completely sunk in the thallus except for the apical group of four cells, which protrudes above the surface. The **venter** encloses an **oosphere** and is surrounded by a jacket of small cells. The **ventral canal cell** may often be seen just above the oosphere, but the neck canal cells have usually gelatinized and disappeared.

Sexual Reproduction : Longitudinal Section of the Sporogonium.—It will not usually be possible to get complete longitudinal sections without the use of a microtome, but sections should be cut to show both the base and the upper part of the sporogonium, as far as possible. Mount in Glycerine.

The base of the sporogonium is enclosed in a sheath, called the **calyptra,** which is formed from the enlarged neck of the archegonium. It extends some distance up from the thallus surface. At the lower end, sunk in the thallus, is the spherical **foot,** which is bounded by several layers of small absorptive cells, in organic continuity with the thallus cells. The centre of the foot consists of large cells which are continuous upwards with a **meristematic zone** of small cells, in which the layer of small **archesporial cells** and the central **columella** are rapidly differentiated. Above this there is a zone of rapid elongation, in which the cells of the columella become drawn out longitudinally and the cells of the archesporium become separated and form **spore mother cells,** in which meiotic division figures may be seen. Many archesporial cells are, however, sterile and remain among the developing spores as strings of more or less collapsed cells, attached to the outer walls of the archesporial cavity. Further up, the tetrads of **spores** are complete and show heavily cuticularized, spinose walls. Towards the top the columella cells become sclerotic and after the dehiscence of the outer wall of the sporogonium by two longitudinal slits, the columella remains as a slender, rigid rod protruding at the top of the opened sporogonium.

Sexual Reproduction : Transverse Section of the Sporogonium.—Cut a transverse section through the upper part of a mature sporogonium. Mount in Glycerine.

The outer layer of cells is cuticularized and forms a protective **epidermis.** The outer wall of the sporogonium is several cells thick and constitutes the **amphithecium.** Within this is the **spore cavity,** in which are the **spore tetrads,** with the small sterile cells among them. In the centre is the sclerotic **columella.** The spores and columella constitute the **endothecium.**

Section 6. Musci

1. ***Mnium.*** *External Morphology.*—*Mnium hornum* is common in woodlands, especially round the base of big trees where leaf mould is plentiful. It forms conspicuous tufts, with stems up to a couple of inches long, set with pointed, translucent leaves, which may be more than 5 mm. long and stand out stiffly all round the stems. A hand lens shows that each leaf has a strong brownish midrib and a narrow brownish margin set with minute teeth.

The stem is dark brown and the lower portion is clothed densely with fine brown **rhizoids,** which make a compact mat at the base of the tuft.

This moss is dioecious, that is the male and female reproductive organs are borne on separate plants. The sex organs are formed at the stem apex in heads called **gametoecia,** surrounded by leaves of a different shape from the others.

The male gametoecium is broad and wide open and is often referred to as a " flower " because it looks like a tiny Daisy. The female remains closed and is not so easily distinguishable except after fertilization, when the **sporogonium** starts to grow from it. The cylindrical, pendent capsule, bright green with a brown lid, is very characteristic and helps to distinguish the species.

> *Transverse Section of Stem and Leaf.*—Transverse sections of the leafy stem, about half-way up, cut in pith and mounted in Glycerine. Sections of the leaves will be produced at the same time, and should be picked out under a hand lens and mounted with the stem sections.

The stem anatomy is very simple. There is no distinct epidermis, the cells of the **cortex** becoming progressively smaller and thicker-walled towards the periphery, while the centre is occupied by a slender core of small thin-walled cells which are elongated longitudinally. This is not a true stele, as it is not separated from the cortex by any endodermis, nor does it contain lignified elements. Its power of water conduction has been shown by experiment to be very limited, and most of the water supply to the upper leaves is drawn from a capillary film of moisture on the stem surface.

The leaves are only one cell thick, except at the margin, which consists of thick-walled elongated cells, and at the midrib, where there is a semicircular bundle of highly thickened fibres. Immediately above the latter is a group of elongated cells, one to two cells deep, which are also thick-walled, but may serve as conductors since they are empty and contain no protoplasm. They do not, however, connect with the core of conducting cells in the stem, but end blindly at the base of the leaf.

> *Longitudinal Section of the Male Gametoecium.*—Look for a plant bearing male " flowers " at the apex of the leafy shoot. These " flowers " or male gametoecia are deep red in the centre and are surrounded by a fringe of leaves, larger than the normal, called the **involucral leaves.** Cut a median longitudinal section through the gametoecium and mount in Glycerine. It may be advantageous to stain in Lactophenol Cotton Blue and mount in Lactophenol, as the antheridia are somewhat transparent.

The stem is expanded at the top into a disc, slightly concave, the middle portion of which is occupied by the **antheridia** and a number of multicellular hairs, the **paraphyses,** which characteristically enlarge towards the upper ends. Their function is uncertain, but they probably secrete water, preventing the drying up of the antheridia.

The latter are cigar-shaped bodies with a single layer of wall cells and contain a mass of minute cubical cells, each of which produces one **antherozoid.** The young stages of the antheridium show how these antherozoid mother cells are formed by the subdivision of a small number of large cells.

The apex of the wall is composed of larger cells which form a cap. These cells separate at maturity and leave an opening through which the antherozoids escape in a mass, embedded in mucilage derived from the unused remains of the mother cells.

> *Longitudinal Section of the Female Gametoecium.*—As the species is dioecious the archegonia must be looked for on plants which show no male "flowers." From such a plant gather one or two stems and cut longitudinal sections through the apical bud.

The leaves which form the apical group are usually larger and of somewhat different shape from the vegetative leaves. They are called the **involucral leaves.** The stem apex which they surround is broadened and flattened and bears a group of **archegonia** mixed with closely packed multicellular hairs or **paraphyses** (Fig. 21).

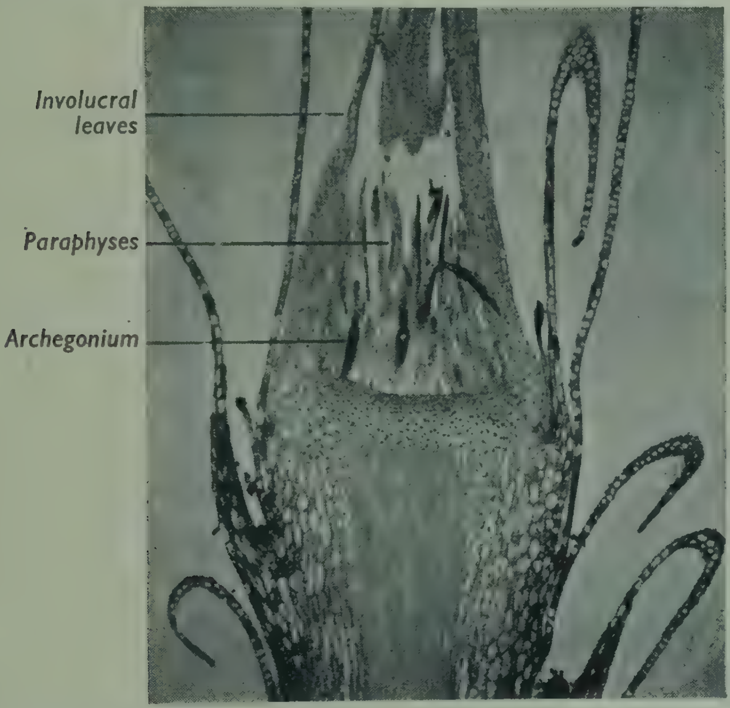

FIG. 21.—*Mnium hornum.* Longitudinal section through apex of female plant.

The archegonia are usually long and massive, with a columnar pedestal or base which is longer than all the rest of the structure and serves to carry up the fertile portion of the archegonium above the paraphyses. The **venter** is very little thicker than the neck, into which it tapers gradually. The

neck canal cells are large and easily seen, and antherozoids may sometimes be seen in the neck canal, looking like little dark curls.

Unlike the antheridia, the archegonia seem to ripen more or less simultaneously, so that they compete with each other. Germinal selection is, however, pretty strict, as it is very abnormal in this species to find two sporophytes from one head.

Structure of the Sporogonium.—The sporogonium consists of a capsule on a stalk or seta, the whole constituting the **sporophyte generation** of the moss.

The **seta** is thin, dark red and stiff, the **capsule** (**theca**) is green, cylindrical and pendent, terminating when fully grown in a nut-brown lid or **operculum.** Loosely covering the young capsule is the pale green **calyptra,** which represents the remains of the parent archegonium. It drops off as the capsule bends over at maturity. When the capsule is mature and the spores ready for dispersal, the outside turns brown and begins to shrivel. Meanwhile the operculum falls off, revealing the **peristome,** a double fringe of teeth attached to the thickened rim of the theca and overarching its open mouth.

Cut off the apex of an old theca and mount it on an opaque background with the peristome upwards, throwing the light on it from above so as to see it by reflected light. Examine with the low power and note the reddish-coloured **ring,** the whitish-coloured **outer teeth** marked with conspicuous transverse bars, and the delicate, irregularly shaped **inner teeth**. These two rows of teeth represent the inner and outer cell walls respectively of a single cell layer, as described in the following section.

The teeth are hygroscopic and tend to curl up in dry weather and vice versa. Thus the mouth is left open for spore discharge only in fine weather.

This property can be very well illustrated by mounting a peristome in a glass cell provided with leading tubes into which moist air bubbled through water or dry air bubbled through Sulphuric acid may be passed alternately. The opening and closing of the peristome teeth may be demonstrated in this way any number of times.

Longitudinal Section of the Capsule.—The capsule should be cut in the young state, when little more than half developed. Older capsules are very difficult to cut by hand and the contents disintegrate easily.

Longitudinal sections show three main portions. At the base is the **apophysis,** which is more or less solid. In the middle is the **theca** or spore capsule proper, while at the apex is the **operculum,** which acts as a lid (Fig. 22).

The apophysis is an expansion of the upper end of the seta. It has an epidermis with strongly cuticularized, domed cells, among which occur sunken **stomata.** These stomata are very like those of the higher plants, except that the guard cells are often somewhat unequal in size and that they are unthickened except round the pore. Beneath each is an intercellular space or **assimilatory chamber** containing small cells rich in chlorophyll. The stomata open and close like those of vascular plants, and their occurrence on the sporophyte but not on the gametophyte of the Mosses is a point to be noticed. The interior of the apophysis is mostly parenchymatous, but there is a central strand of narrow elongated cells which continues downwards through the seta.

The parenchymatous part of the apophysis continues directly upwards to form the central **columella** in the theca, around which lies the cylindrical layer of **archesporium,** which later develops into cuticularized spores. Between the archesporium and the outer wall of the theca is a space, bridged over by a few strands of thin-walled cells which act as links to hold the parts together. These disappear as the spores mature, and the columella likewise breaks down, so that the spores are left loose in the interior of an otherwise empty theca.

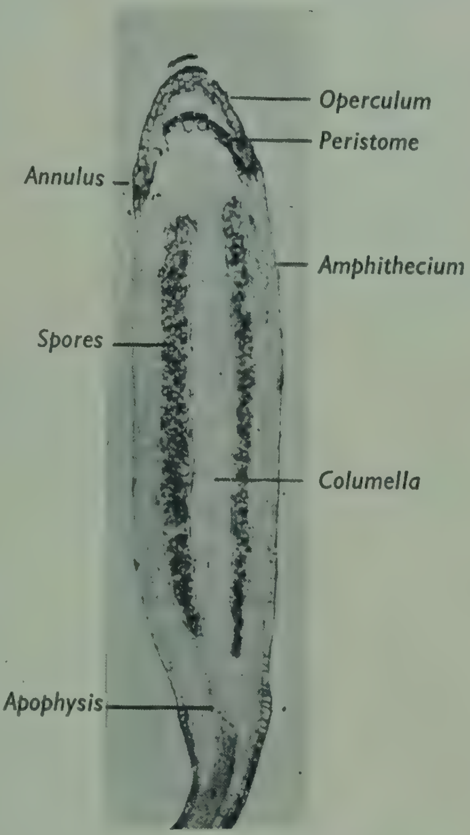

FIG. 22.—*Mnium hornum.* Longitudinal section through capsule.

Over the top of the theca stands the solid dome of the operculum. Where this joins the theca wall there is intercalated a ring of two or three rows of large thin-walled cells which contrast strongly with the highly thickened cells above and below. These cells are highly vacuolated, and it is their osmotic swelling which ruptures the connection between the theca and lid at maturity. They are overarched by the thickened cells above and below them, so that they are invisible from the exterior and are thus protected from desiccation. This important layer has been compared to the **annulus** of the fern sporangium. It serves a similar purpose and is called by the same name.

The thickened margin of the theca is called the **ring.** It extends inwards to the base of the peristome teeth, which begin to form under the dome of the operculum. They represent, as it were, an inner dome, which is formed from one layer of cells, thickened with yellow cuticle both on the outer and inner walls, but with very thin portions on the cell walls, which soon disappear, leaving the two layers of thickened side walls separate. This inner dome splits radially into a number of separate teeth in two concentric layers, and when the top of the operculum eventually falls off, the teeth are left forming two rows overarching the open mouth of the theca and free to hinge on the ring, to which they are attached at their base (Fig. 23).

Protonema.—The protonemata of nearly all Mosses are exceedingly similar, and almost any one may be used as a type. Mount in Glycerine.

The protonema consists of branched filaments of cells with many discoid chloroplasts. The most easily recognizable peculiarity is the oblique position of the cross walls between the cells, which easily distinguishes moss protonemata from filamentous algae of similar appearance.

Sometimes the walls are reddish coloured, especially in persistent protonemata which last throughout the life of the leafy gametophyte. The leafy shoots arise from lateral outgrowths of the protonema cells, which are cut off by two transverse walls intersecting at an angle of 120°, and thus isolating the apex of the outgrowth as a more or less pyramidal cell, which functions as the apical cell of a new leafy shoot.

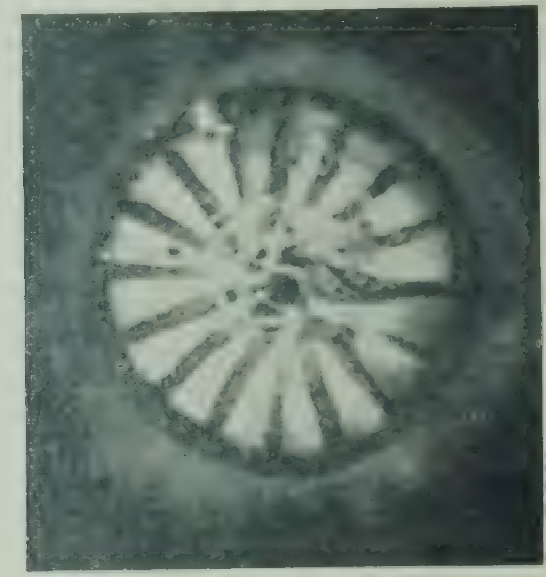

FIG. 23.—*Mnium hornum.* Structure of the peristome.

2. **Funaria.** *External Morphology.*—Tease out a small tuft of leafy shoots of *Funaria hygrometrica* under a dissecting lens, preferably in a shallow dish with a little water.

The tuft is composed of leafy stems about 1 cm. long, which increase in thickness upwards towards the apex. Most of the leaves are gathered into an apical tuft, and the lower part of the stem is buried in a tangled mass of brown **rhizoids.** Loosely attached branches spring from the upright stem, some short and some long and delicate. The latter creep horizontally to extend the tuft.

The leaves are broad and translucent. They have no thickened margin, but there is a conspicuous **midrib** which is prolonged at the apex into a long sharp point or **apiculus.**

On the male plant the leafy shoot ends in a **male gametoecium** containing numerous **antheridia,** with enlarged **involucral leaves** surrounding them. These gametoecia look like minute flowers and are often referred to as such, though, of course, they are really gametophyte structures and have nothing to do with the flowers of the higher plants.

On female plants the leafy shoots end in the **female gametoecia** surrounded by **involucral leaves** containing a small number of **archegonia** with exceptionally long necks. Only one of these produces a **sporophyte,** though in some other mosses multiple fruit production is common. The **sporogonium** is provided with a long arcuate (*i.e.*, bowed) seta and a pear-shaped spore capsule which has the **operculum** set obliquely, so that it is unsymmetrical. This is unusual and detracts somewhat from the value of *Funaria* as a type. At the foot of the seta may be seen a minute, short sheath, which is formed by the upgrowth of gametophyte tissue, enclosing the base of the seta. This is the **vaginula,** and it serves to maintain the attachment of the sporophyte to the gametophyte plant.

The young capsule is partly covered by a loose membranous cap, usually split down one side, with a long point. This is the **calyptra,** representing the remains of the archegonium in which the embryonic sporophyte was developed.

Transverse Section of Stem.—The stem of *Funaria* is delicate and minute, and its anatomy correspondingly simple.

Transverse sections show a **cortex** which is made up externally of thick-walled cells and of thin-walled cells towards the centre, with a gradual transition between. There is no distinct epidermis. In the centre is a group of small " conducting cells," elongated longitudinally, but with thin unspecialized walls. In some sections one may also see one or more groups of similar small cells in the cortex. These are **leaf trace bundles,** but they do *not* connect with the central bundle and end blindly in the cortex not far below the leaf base from which they came.

Male Gametoecium.—The male " flower " is very like that of *Mnium* (p. 118), except that it is smaller.

The stem apex is scarcely expanded, and the number of antheridia is therefore considerably less than in the former type. The paraphyses are elongated upwards, but they end in a globular cell instead of a pointed one. The antheridia are smaller but otherwise similar, and so are the involucral leaves.

Female Gametoecium.—The archegonial groups are formed at the apices of leafy shoots and are in all essential respects exactly like those of *Mnium* (p. 119).

Longitudinal Section of Capsule.—The longitudinal section must be cut from an immature sporogonium, otherwise nothing will be seen of the internal tissues. The general arrangement is very similar to that of *Mnium* (p. 120).

The **apophysis** is long and tapers downwards gradually into the **seta.** There is practically no central strand such as *Mnium* shows, but there is, on the other hand, a well-marked spongy tissue below the epidermis, very like that of an angiospermic leaf. The epidermis is relatively thin-walled, and the stomata are not sunken.

These facts are peculiar in as much as the habitat of *Funaria* is on heaths, in much drier situations than *Mnium*, and one would expect it therefore to be the better protected of the two against the effects of drought. The **theca** has a large-celled **columella** in the centre surrounded by the cylindrical **archesporium** layer and later by the **spore layer,** which develops from the archesporium. Around this is a space traversed by fine strands of chlorophyll-containing cells. These, however, shrivel and disappear before maturity, leaving the theca wall and the spore layer unconnected. The columella is joined at its base to the tissues of the apophysis, but the junction between them is contracted into a narrow isthmus.

The ripe spores, if present, are furnished with a thick yellowish-brown cuticle and look like minute pollen grains.

The arrangements in the operculum are closely similar to those of *Mnium*. The **annulus** layer is larger and even more distinct, but the **ring** is less developed. The **peristome teeth** are formed in the same manner from the thickened inner and outer walls of a single layer of cells, which become disjoined from each other to produce an outer ring of large teeth marked with transverse bars and an inner ring of more delicate teeth. The action of the peristome teeth is similar to that in *Mnium* (see p. 120).

If longitudinal sections are cut through the lower end of the seta at an early stage it will be seen that the base of the archegonium surrounding it has been expanded to form a loose bag which contains water, thus protecting the young embryo from desiccation, a function analogous to that of the "water calyces" of some angiosperms.

3. ***Polytrichum.*** *External Morphology.*—There are numerous species of *Polytrichum* in Britain and some of them are very abundant on heaths (*e.g.*, *P. juniperinum* and *P. piliferum*), but by far the biggest and best developed is *P. commune*, which, in spite of its name, is not the commonest. It grows chiefly on boggy ground among grasses, where it forms deep cushions from which stems more than a foot in length may be pulled out. With one exception it is the largest known moss.

The stem is thin and wiry, brownish in its lower portion, and it is closely set with narrow, pointed leaves about 1 cm. long, the youngest of which are pale green. The apical leaves form a pointed group, but are not closely associated into a true bud.

The basal part of the stem is underground and is devoid of true leaves, though bearing leaf scales without chlorophyll. The lowest part is called a **rhizome,** and it is covered with a thick coat of brown tangled **rhizoids,** but has no leaf scales. Anatomically, also, it differs from the leafy stem, as described in the next section.

In the male plant the stem ends above in a rather conspicuous **gametoecium** with reddish-coloured **involucral leaves** or bracts enclosing it. In this are the numerous large **antheridia,** but in the centre there is also a dormant growing point which often starts to grow in the autumn, forming a new stem which ends in a second gametoecium, and so on; thus a succession of three or four may be produced, with a year's growth of 2 or 3 in. of stem between them.

The female plant is similar to the male, but the stems end in archegonial clusters surrounded by **involucral leaves,** which are not notably different from the others. The female gametoecia are not open like the males, and not easily discovered except by dissection. From them grow out singly the sporophytes (**sporogonia**). There is no proliferation as there is in the males.

The **seta** is sturdy and may be several inches long. It ends in a spore-capsule which is broadly four-angled, widest at the base and surmounts a small ring-like **apophysis.** There is a sharply pointed **operculum** joined to the capsule by a bright red **ring.**

The whole spore capsule is usually covered until ripe by a remarkably large **calyptra** covered with hairs, from which the genus takes its name of *Polytrichum*, or " many haired."

Transverse Section of Stem.—Transverse section near the top of the leafy stem. Stain with Iodine and mount in Glycerine.

The anatomy of the *Polytrichum* stem is somewhat complex and confused, as the various tissues which have been distinguished by research workers have not got the clear limits shown by most of the tissues of higher plants. This is a natural consequence of the relatively lowly status of the plant. Nevertheless, the stem shows most interesting similarities to the true vascular sporophytes, and an attempt will therefore be made to describe at least the main features.

The main stem and the rhizoid-bearing rhizome below ground differ considerably, and there is a long transition region between them, so that it is necessary that we should concentrate on a section taken high up on the leafy stem and a section as far down as possible in the underground portion.

Aerial Leafy Stem.—Externally there are one or two layers of small, very thick-walled cells which merge into a thickened **cortex** (Fig. 24). Scattered through this are curved groups of thin-walled cells, the **leaf traces,** which pass out into the leaf midribs.

The centre of the section is occupied by a solid group of rounded cells with walls which are partly very thick and partly very thin. These are elongated empty cells, probably for the conduction or storage of water. Their ends are pointed and overlap each other, and the thin walls are those between such overlapping ends. As there are no pits on the walls these thin walls probably facilitate the passage of water. These cells are called **hydroids,** with reference to their conducting function. Around them lies a zone of cells similar in shape and apparently in function, but with thin walls.

These cells are also hydroids, and together with the thick-walled hydroids they form the **hadrome** tissue.

Next comes a layer of smaller and thicker-walled (suberized) cells which contain starch grains and are referred to as the **amylome.** They are dark-coloured in Iodine. Outside of this is a zone, not altogether complete in some cases, of light-coloured, thin-walled cells, the **leptoids,** forming the tissue called the **leptome,** to which a phloem function has been assigned. Outside this leptome mantle we come once more to the cortex.

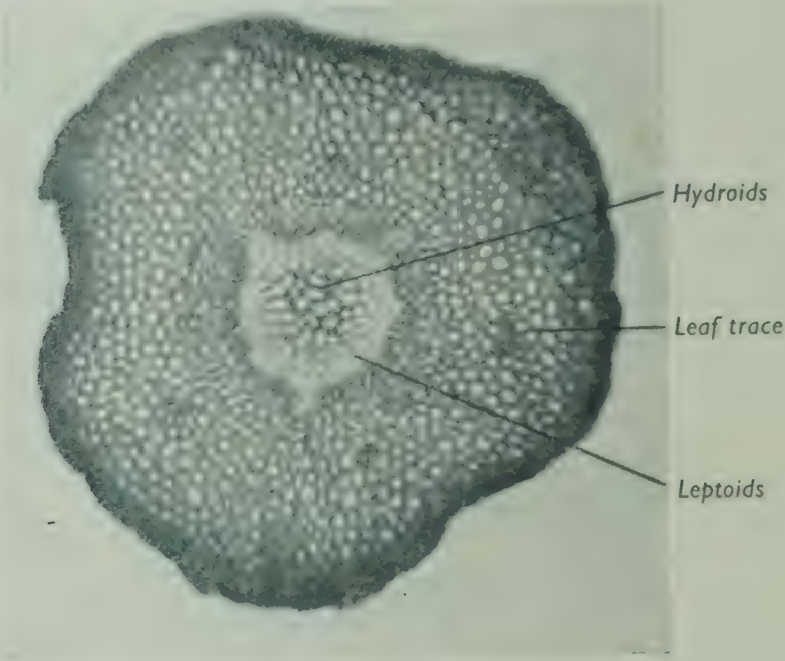

FIG. 24.—*Polytrichum commune.* Transverse section of aerial stem.

Subterranean Rhizome.—The centre of the section is occupied by a roughly triangular mass of hadrome in which there is a relatively small number of hydroids and a large number of thick-walled fibre-like cells or **stereids** (Fig. 25). These latter are absent from the hadrome of the upper stem. The hadrome mass is furrowed by three deep grooves, opposite to each of which, externally, is a wedge of thick-walled fibre-cells, pointing inwards. Below the point of each wedge, and inside a groove of the hadrome, there lies a small group of thin-walled cells with cytoplasm and starch grains. These represent the leptome zone of the leafy stem.

Surrounding the hadrome and interrupted only by the above-mentioned wedges, there is a conspicuous zone of large rectangular cells, the *lateral* walls of which are suberized, though their inner and their outer walls are not. This gives it, in fact, the character of an **endodermis,** which is not present in the leafy stem. Around this is a narrow zone of thin-walled cortex and an equally narrow zone of thick-walled cells which forms the periphery, and from which arise the **rhizoids,** brown coloured and thick walled, in which the rhizome is enveloped.

The most notable thing about the anatomy of the rhizome is that the leptome lies between the lobes of the hadrome, in the same way as the phloem lies between the protoxylems in the roots of higher plants. In the stem, on the other hand, the leptome is arranged concentrically with the hadrome, as is the xylem and phloem of angiospermic stems.

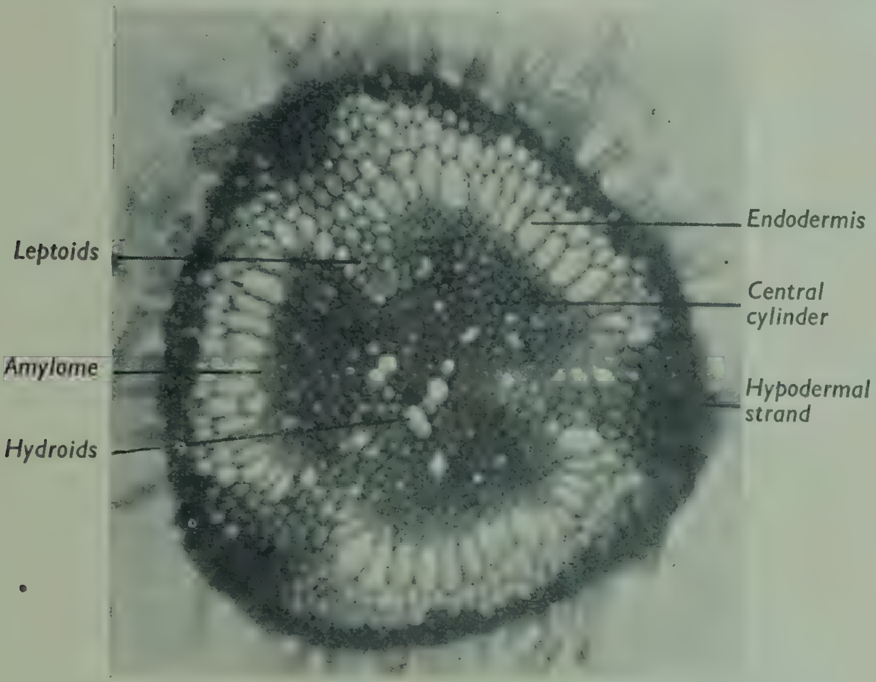

FIG. 25.—*Polytrichum commune.* Transverse section of rhizome.

> *Transverse Section of Leaf.*—The leaf has two distinct portions, a narrow pointed blade and a broad membranous leaf-base with two wings surrounding the stem. Cut a transverse section through the blade portion and mount in Glycerine.

In the section the upper surface of the leaf appears to be thickly covered with short upright filaments of cells. These are really cross-sections of the **lamellae,** running longitudinally, which cover the leaf surface from edge to edge. They can be seen in the fresh leaf with a powerful hand lens. Each lamella is six to seven cells high, and the cells of the upper margin have two divergent ridges on their upper sides. The shape and position of these ridges varies in different species, and is one of the chief means of distinguishing them when sterile. The chloroplasts are confined to the lamellae and are absent from the leaf blade.

The blade of the leaf itself is thin and its edges are turned up side by side with the outermost lamellae. The edges are made up entirely of sclerotic fibre-cells, but the midrib shows a layer of thin-walled conducting cells (hydroids), between two layers of fibres, above and below.

In *P. juniperinum*, which grows in dry places, the margins of the leaf extend widely beyond the lamellae and fold back over the top of them protectively in dry weather.

> *Male Gametoecium.*—Cut a longitudinal section through the enlarged antheridial head of a stem and mount in Glycerine.

The apex of the stem enlarges somewhat into a disc of very delicate cells from the surface of which arise the **involucral leaves.** In the centre is a minute **apical bud,** and around this are the antheridia, interspersed with long delicate **paraphyses,** which overtop the antheridia and form a compact surface of somewhat thickened cells above them. The antheridia are produced in a prolonged succession so that they may be seen in various stages of development. Each **antheridium** possesses a long tapering stalk or pedicel. A curious and unexplained point is that each stalk is traversed from side to side by a layer of suberized cells which develop while the antheridia are still quite young. As this presumably cuts it off from the water supply from the stem, it must absorb water from the paraphyses or from raindrops.

The antheridia are much larger than in *Mnium* or *Funaria*, but they have exactly the same type of wall and of contents and they dehisce at the apex to release the ripe **antherozoids.**

In transverse sections of the gametoecium it may be seen that the antheridia are arranged in a number of separate groups, each in the axil of an involucral leaf. The latter are not in one whorl but are spirally arranged, so that the whole structure of the gametoecium is rather that of an " inflorescence " than of a " flower."

> *Female Gametoecium.*—The archegonia are to be found at the apices of stems which bear no male gametoecia, and are not provided with sporogonia. A number of sterile apices occur, so that several may have to be cut in order to find a female head. They should be looked for in early June.

The **involucral leaves** are indistinguishable from the vegetative leaves, and they are not expanded like those on the male head but overlap closely, so that the small group of apical archegonia is very well enclosed.

The **archegonia** are massive and have a substantial base or pedestal. The hairs which later cover the calyptra begin to develop very early on the archegonial wall, and may be seen even before fertilization. They provide a very distinctive feature.

It has been suggested that in their youngest stages these hairs absorb moisture.

> *Longitudinal Section through Capsule.*—Choose an immature capsule from which to cut a longitudinal section, otherwise the interior will show nothing but spores. A fairly thick section is no disadvantage. Mount in Glycerine.

The three portions of the sporogonium are sharply distinct. The **apophysis** is relatively small and entirely parenchymatous, the outer layers being brownish and apparently cuticularized. It is separated from the theca by a relatively narrow neck, on which are the stomata, which are thus protected by the outgrowth of the tissues above and below them.

The **theca** has an outer layer (**amphithecium**) of considerable thickness and with a very heavily cuticularized epidermis. The **columella** is narrow, but the spore layer on the other hand is exceptionally massive and so extensive that it is thrown into wide folds. It is separated from the amphithecium by a space in which may be seen numerous transverse filaments containing chloroplasts.

At the upper end the columella expands and is surmounted by a thick parenchymatous disc, the **epiphragm,** which extends right across the capsule. At the edge of the epiphragm is the **ring,** of reddish coloured cells, attached to which are the delicate bundles of sclerotic cells that here represent the **peristome teeth** (Fig. 26). The **operculum** early becomes detached from the epiphragm at the centre, and its shrinkage, as it dries at maturity produces a vacuum which draws the ring inwards until it ruptures, whereupon the operculum is shot off by the entering air.

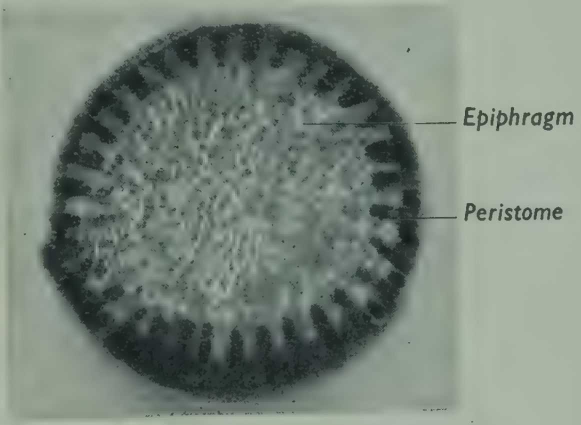

FIG. 26.—*Polytrichum commune.* Structure of the peristome.

The epiphragm, separated all round from the external tissues, acts as a controller of spore discharge. In dry weather it contracts, allowing spores to escape, but in damp weather it expands and fits closely against the ring, thus closing the capsule.

4. ***Sphagnum.*** *External Morphology.*—Examine a single plant, if possible in the fruiting condition. There are many species of *Sphagnum* but they resemble each other closely in essential particulars.

There is a very slender main **stem,** which bears a large number of **leafy branches** which arise from the axils of leaves on the main stem. The lower end of the stem is usually decayed because *Sphagnum* has indefinite powers of growth upwards, leaving behind a peaty substratum of decayed remains. If young plants are available they will be seen to arise from a foliose **protonema,** which persists for a considerable time.

The branches are of two kinds. The lower branches are usually long and slender, with widely spaced leaves, but those near the apex are generally closely set, short and stout, with overlapping leaves, forming a sort of crown at the top of the stem. It is on these apical branches that the sex organs are borne and from some of them the **sporogonia** arise. These take the form of spherical **spore capsules** of a chestnut brown colour, borne on short colourless stalks called **pseudopodia.** The pseudopodia are leafless prolongations of the gametophyte axis. They are not setae comparable with those of the Mosses, which are part of the sporophyte. The **foot** of the capsule is very short and is embedded in the top of the pseudopodium. If the capsules are ripe they will show a round **operculum** on top, by means of which the spore capsule opens.

> *Transverse Section of Stem.*—Cut a transverse section through the lower part of the most robust piece of stem available. Mount in Glycerine.

The stem structure varies a good deal in different species of *Sphagnum*, but a well-developed stem should show the following features. Externally there is a zone of **cortex,** consisting of large squarish cells, devoid of contents. The walls of these cells are perforated by large round **pores,** which connect the cells to each other and to the outside. The tissue is therefore spongy and absorptive and is comparable physiologically to the velamen of an Orchid root. In some species large **flask cells** are formed in the cortex, with a short neck, projecting outwards. Sometimes Rotifers may be found living in these cells. Inside the cortex is a zone of thick-walled **hadrome** with small cells, and in the centre is a thin-walled **medulla,** the cells of which are much elongated longitudinally and may have a conducting function.

> *Structure of Leaf.*—Select some leaves from a branch and mount in Glycerine.

The leaf structure is unique. The chloroplasts are confined to a number of long narrow cells, so arranged that they form a network, the meshes of which are filled by large, colourless, empty cells. These latter cells are girdled by prominent rings of cuticularized material, which apparently support the walls. The walls are perforated by large **pores,** communicating with the outside, so that these cells also are absorptive. There are no special marginal cells and no midrib. The whole leaf is only one cell thick. Towards the base and the apex of the leaf the cells become thick-walled and devoid of rings, but this is the only differentiation of structure.

> *Sexual Reproduction : Antheridia.*—The antheridia are borne on short branches near the apex. The antheridial branches may usually be recognised by their swollen, club-shaped appearance. Tease out in Glycerine.

Each **antheridium** arises from the stem just above a leaf axil. It has a long, thin stalk and is itself large and ovoid in shape. There is a **wall** consisting of a single cell-layer, the cells containing **starch grains** and enlarging towards the apex of the antheridium. At maturity the wall at the apex splits into a number of flaps, which roll backwards, leaving a wide opening for the release of the antherozoids.

Sexual Reproduction :—Archegonia.—The archegonial branches may be on the same plant as the antheridial branches or on different plants. They are stout, short, and sharp pointed and are always close to the apex of the stem. The leaves are large and sheathing. Tease out a branch in Glycerine.

The leaves of the archegonial branch are called **involucral leaves.** They are closely wrapped around the branch, forming a sheath. Their cells are more elongated than those of the foliage leaves. The apex of the branch is swollen and cushion-like and bears a group of three **archegonia,** with long necks and very short stalks. Only one finally develops into a capsule, which is at first enclosed in a **calyptra** formed of the enlarged venter of the archegonium. The foot of this capsule is embedded in the swollen stem apex.

Sexual Reproduction : Longitudinal Section of the Sporogonium.—Cut longitudinal sections of the young spore capsule and foot neglecting the pseudopodium. Mount in Glycerine.

The capsule **wall** consists of an outer layer of more or less cubical cells with cuticularized walls, which become dark coloured when mature. Within this is an inner wall of three or four layers of thin-walled cells. From the base of the capsule arises a dome-shaped mass of thin-walled cells, the **columella.** Over the top of the columella is the mass of **spores,** forming a hollow dome. The spores are yellow when ripe and have thick, rough walls. They are grouped in **tetrads** and each is marked with a tri-radiate ridge where they are in contact with the other spores of the tetrad. The whole mass is enclosed in a thin but tough membrane.

At the top of the capsule part of the outer wall is marked off as the **operculum,** around which runs the circular **annulus** of small, thickened cells, along which the dehiscence of the capsule takes place. At the base of the capsule is the **foot,** formed of delicate cells, which is enclosed within the cup-shaped upper end of the pseudopodium. This cup is lined with small cells, with dense contents and large nuclei, from which soluble food materials are transferred to the sporogonium.

Section 7. Filicales

1. **Dryopteris.** *External Morphology.*—Dig up a mature plant of *Dryopteris filix-mas*, the Male Fern, and, after washing away the superfluous soil, examine the specimen.

The plant consists of a short conical **rhizome** from which a number of separate leaves arise. These leaves, or **fronds,** each consist of a petiole which is brown in colour at its lower end and covered with golden-brown scales or **ramenta** farther up. The petiole passes imperceptibly into the **rachis,** which bears **pinnae** in two rows, the individual segments being inserted almost, but not quite, opposite one another. Each pinna contains a central vascular strand which gives off branches into smaller lateral laminae which form the **pinnules.** On the lower surface of these pinnules are the **sori,** borne in two rows. It will be noticed that not all the fronds bear sori, so that we may separate sterile from fertile fronds.

Each frond is attached at its proximal end to the rhizome, and at the point of insertion, adventitious roots arise which grow downwards and cover the rhizome. Since the petioles are produced in acropetal succession the rhizome becomes clothed with roots, for these persist even after the leaves have dropped off. At each such point the base of the petiole persists indefinitely, forming an armour of leaf-bases round the stem.

The fronds are developed from the base of the stem or rhizome forwards, so that the growing apex of the rhizome is covered over with young fronds. These fronds are curled up, that is to say, they exhibit **circinate vernation.** In addition to the adventitious roots the upper part of the rhizome is also covered with ramenta.

In the earliest stages a short primary root is developed, but this very soon disappears and its function is taken over by the adventitious roots.

Dissected Stele of the Stem.—A young rhizome is selected, the leaf-bases are cut away and the rest of the rhizome is placed for twenty-four hours in a mixture of one part of Hydrochloric acid and three parts of 95 per cent. alcohol and then gently warmed in ½ per cent. ammonium oxalate for an hour or two. When the tissues have become partly disintegrated it is possible, with care, to dissect away the sclerenchyma and cortical tissues, leaving only the stelar system intact.

An examination of such a stele will show that it is roughly conical in shape and that it consists of a network of **meristeles** which give off externally numerous short strands which pass out to the petioles (Fig. 27). The number and complexity of these lateral strands increases as one passes from the base towards the apex of the system, but they are mainly concentrated round the lower sides of the openings, which are called the **leaf gaps.**

Transverse Section of the Stem.—Owing to the condensed nature of the stem of the Male Fern it is difficult to make out the structure in a transverse section, though the general arrangement of the parts may be deduced from a study of the dissected stele already referred to. From a histological standpoint it is usually found best to make use of the rhizome of *Pteridium aquilinum* (Bracken Fern), which is more elongated and the parts are therefore easier to elucidate (see p. 140).

Transverse Section of the Petiole.—A transverse section taken through the petiole of a frond and stained in Aniline chloride will show the following features.

The outline of a section of the petiole is roughly circular, but the upper or adaxial surface is slightly flattened, and in this flattened region there is a definite groove running up the petiole. By means of this the section may be correctly orientated.

Bounding the outside of the section will be seen a number of layers of heavily lignified cells which form a thick outer covering to the petiole. The rest of the ground tissue consists of thin-walled parenchyma which forms a continuous tissue without any intercellular spaces. Embedded in the ground tissue are a number of segments of the stele (meristeles) which are arranged in the form of a horseshoe with the two largest steles at the ends.

Each meristele is surrounded on the outside by an **endodermis** composed of rectangular cells with the inner tangential walls thickened with dark brown material which spreads a short way round on to the radial walls. Inside the endodermis is the **pericycle,** made up of a single layer of more rounded cells. The central tissue consists of a mass of **xylem.** There are two **protoxylem** groups, one at each end, lying close to the pericycle, and between them is the **metaxylem.** On either side of the xylem, bands of **phloem** composed of small whitish-walled cells, some of which show sieve plates, are clearly visible. There is a small quantity of **xylem parenchyma** between the xylem and phloem. All the meristeles have the same general structure.

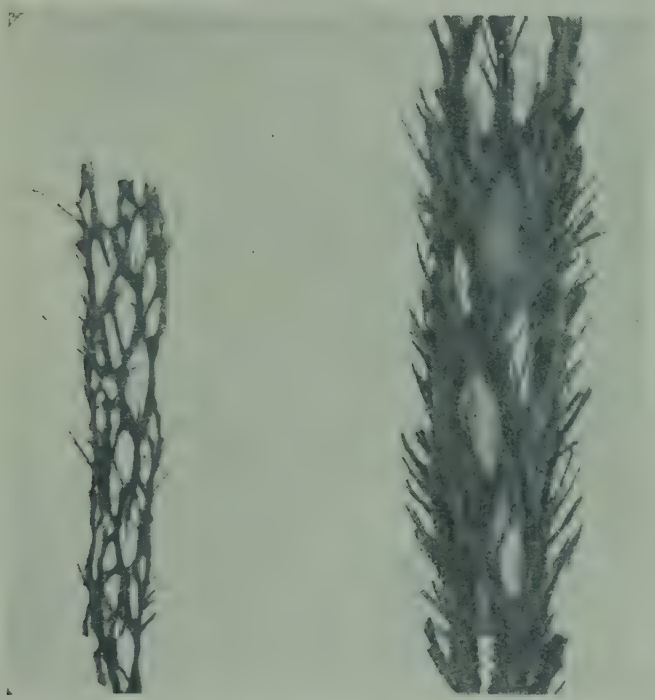

FIG. 27.—*Dryopteris filix-mas.* A dissected stele.

Surface Section of Leaf.—Strip off a small piece of the lower epidermis from a pinnule by bending it back and then tearing carefully. Plunge the piece immediately into absolute Alcohol to fix the stomata and mount in pure Glycerine or liquid Paraffin.

The cells of the epidermis are more or less rectangular, with very deeply lobed margins which fit into one another like pieces of a jig-saw puzzle. Scattered irregularly over the surface are the **stomata.** Each stoma consists of two **guard cells,** the outer walls of which are convex, so that the two guard cells together form an oval. The walls next to the pore are thin and the guard cells, by contraction or expansion, control the stomatal pore, by which air enters the leaf. Unlike the other epidermal cells the guard cells are filled with chloroplasts. If the slide has been hurriedly prepared it may appear at first sight that there are chloroplasts in the epidermal cells, but more careful observation will show that these chloroplasts belong to cells of the **mesophyll** lying below the epidermis, which have remained partly attached to the epidermis in the stripping. It should be noticed that both in the structure of the epidermal cells and also in the morphology of the stomata the leaf of the fern differs little from that of the higher plants.

> *Transverse Section through the Root.*—The roots of a Fern are of two kinds. First, there is the short primary root which is only present in the very young state. Secondly, there are the adventitious roots which arise from the base of the petioles. These roots are very slender and must be cut in pith or Carrot to obtain good sections. Stain in Aniline chloride and mount in Glycerine.

On the outside will be seen the remains of the outer **cortex** which soon disorganizes. The inner cortex consists of heavily thickened cells which are yellowish-brown in colour. Two or three of the innermost layers are by far the most highly thickened and also consist of the smallest cells.

Internal to the cortex lies the relatively thin-walled stelar tissue, surrounded by a very thin-walled **endodermis** and **pericycle,** which may become partly disorganized. Inside this lies the central **metaxylem** consisting of large tracheids, with two **protoxylem** groups, one lying at each end and immediately adjacent to the pericycle. On either side of the metaxylem and filling in around it are the small cells of the **phloem.** It will be noted that the amount of phloem is very small in comparison with the xylem. The whole of the cells of the cortical zone are heavily impregnated with tannin.

> *The Sorus: General Features.*—Examine the lower surface of a fertile frond with a hand lens.

The sori are arranged irregularly in two rows along the pinnules, there being generally about six on each pinnule. The sori are greyish-green in colour when young and each is more or less reniform in shape with a depression in one side. They are covered by an **indusium.**

In the older part of the frond the indusium will be seen to have withered and rolled up, displaying the brown **sporangia** which have been protected by it during their development.

It will be noticed that a branch of a vein may be seen associated with each sorus, whereby food for the development of the spores is obtained.

Transverse Section of the Leaf and Sorus.—Cut a transverse section through a young pinnule bearing a sorus on its lower surface, stain with Aniline chloride and mount in water.

The section shows the more or less rounded cells of the upper epidermis, below which are successive layers of mesophyll cells containing chloroplasts. These cells are arranged very irregularly, and large intercellular spaces are left between them. The lower surface of the leaf is bounded by a second epidermis of similarly shaped cells.

In the region of the sorus lies the end of a vascular bundle consisting of xylem and phloem, embedded in a mound of closely set parenchymatous cells. From the lower surface of the **placenta** so formed, elongated colourless cells arise in a column which spreads outwards and bears the **indusium,** a kidney-shaped membrane composed of a single layer of thin-walled colourless cells, which in section appears as two curved lines of cells overarching and enclosing the placenta. From the sides of the placenta arise the sporangia. Each sporangium is borne on a multicellular stalk which terminates in the sporangium itself and bears laterally a **glandular hair.**

The **sporangium** is a flattened structure with two sides formed of thin-walled cells which are polyhedral in shape. Between these is developed the **archesporial tissue** from which the spore mother-cells are formed. Following the reduction division of the nuclei of these spore mother-cells, of which there are some sixteen, the **spores** are produced, there being thus about sixty-four spores formed in each sporangium. The whole sporangium when mature is brown in colour. Around the edge of the flattened sporangium lies the **annulus,** composed of very uniform cells whose inner tangential and radial walls are greatly thickened, while the outer tangential wall remains thin. On one side, between the beginning of the stalk and the end of the annulus is a small group of long flat cells which are thin-walled and are termed the **stomium.** It is at this point that the splitting of the sporangium begins.

All the sporangia are not of the same age, but they are developed on the placenta successively. Thus in a single section sporangia at different stages of development can be studied. Occasionally two sporangia may develop from a common stalk.

When shed, the spores are seen to be very thick-walled. The outer layer is very rugged and almost black in colour.

Dehiscence of the Sporangium.—Soak some small pieces of a fern frond, possessing sori, in water for a few hours. Remove and carefully dry off the surplus water with filter paper and mount some sporangia dry and uncovered on a microscope slide. Examine under the low power of the microscope, illuminating the object from above by means of an electric lamp fixed close to the microscope so as to provide considerable heat as well as light to the slide. In this way the sporangia will be heated up and dried. It will only take a few minutes before the sporangia have dried sufficiently to split open.

After a short while the first sporangium will suddenly spring open, splitting irregularly along the flat side, exposing the spores, some of which will be thrown clear and out of the field of view. This splitting is brought about by the contraction of the cells of the annulus, which exerts such a strain on the thin-walled cells of the stomium that they finally break, and the split continuing across the sides of the sporangium, bursts the whole open and throws out the spores. If the mount is now treated with a drop of water it will be seen that the walls of the annulus cells expand once more, and the sporangium closes more or less as it was, when, by again heating, the opening of the sporangium may be repeated. The contraction of the annulus is due to cohesion of the water in the cells to the substance of the thickened walls. As the water diminishes in drying, the walls are drawn together. If drying is continued after the splitting of the sporangium a point is reached at which the cohesion breaks down, air enters the cells and the sporangium suddenly closes once more. Since all the movements are jerky any spores still attached to the sporangium will ultimately be shaken out.

Another method of observing the dehiscence of the sporangia is to mount them, straight from the sorus, in strong Glycerine. This withdraws water from the annulus cells and causes them to contract and to break open the sporangial wall. The viscosity of the Glycerine prevents the spores from being ejected for more than a short distance.

> *Germination of the Spores.*—The spores may be easily germinated by keeping them on a piece of moist filter paper in a Petri dish.

First the spore wall splits and there grows out a short filament of rounded cells. These cells soon develop chloroplasts. Very soon, as a result of division of the cells longitudinally, a flat plate of tissue is produced. Meanwhile, from the end to which the spore coat was attached, **rhizoids** will appear as single-celled brown threads. More rhizoids develop, and by the time the **prothallus** is about 2 mm. long, a median cushion has been formed by further division of certain central cells, to produce a mass of tissue up to about six cells thick. It is from this tissue that the sex organs arise. As the prothallus becomes completely organized it assumes a heart shape with a deep depression at the apical end, in which lies the apical cell from which new cells are cut off.

> *Mature Prothallus.*—Mount a young prothallus, about 5 mm. across, with the lower surface uppermost.

The young gametophyte will be seen to consist of a heart-shaped structure composed of rather elongated polygonal cells, densely filled with small discoid chloroplasts. The margin is slightly irregular, but there are no special marginal cells. The structure is one cell thick with the exception of a small cushion of tissue lying in the central line, immediately behind the growing apex. Arising from this central tissue or **cushion** are numerous elongated cells, brown in colour, thin-walled and quite unbranched. These are the **rhizoids.** They are absorbing and attaching organs. It will be found that if the prothallus was growing in soil many soil particles are attached to these rhizoids. Among the rhizoids, near the apical end, will be found the **archegonia,** which will be seen either as spherical organs consisting of a ring of four cells enclosing a cavity, or as a tube of about four cells lying on the surface of the thallus. These different appearances are caused by the fact that while young the archegonia grow vertically out from the lower surface of the cushion, and will therefore be seen from above, but later, as they mature, the necks of the archegonia bend over and will then be seen from the side.

Farther back on the thinner part of the cushion near the base of the prothallus will be seen the rounded **antheridia,** each consisting of a central cell, surrounded by a wall of thin cells and covered by a cap-cell. When ripe the central cell contains minute coiled **antherozoids,** which are released by the detachment of the cap-cell. Here and there on the edge of the thallus are small stalked **glands** each of which terminates in a small globular cell. These glands are often mistaken for antheridia.

In a slightly older prothallus, about 7 mm. across, it will be found that only remains of the antheridia can be seen, while the archegonia have elongated, the central canal in the neck has become more pronounced and the four lip cells at the apex of the neck have separated, leaving an opening. The canal may also be seen to have extruded a liquid, and the colour of the opening has changed from green to brown. This is the fertilization stage. The rhizoids have become more numerous and individually longer while the edge of the prothallus is more frilly, due to uneven enlargement of the marginal cells.

> *Longitudinal Section of the Antheridium.*—A good hand section taken through the growing apex and along the cushion may show the development of the antheridium. More satisfactory results will be obtained by using prepared slides made from a microtome ribbon.

In such a slide will be seen the thin wall of cells, two cells high, which forms the wall of the antheridium, and the dome-shaped cap-cell on top (Fig. 28). Inside lie the **antherozoids,** which stain deeply. About a score may be seen in any section. There are actually about sixty-four in each antheridium. Such a section will also show the multicellular character of the cushion and the unicellular thickness of the rest of the prothallus.

> *Longitudinal Section of the Archegonium.*—A section of a mature prothallus, similar to that described in the last section, may be used to show the structure of the archegonium.

The archegonia lie towards the front end of the cushion, in a region where the prothallus may be as much as six cells thick. Each **archegonium** consists of a **neck** and a **venter** (Fig. 29). The neck is four cells high, and each tier is made up of four cells. In the early stages the apex of the neck is closed, but as the archegonium matures, the contents of the neck canal disorganize, swell and push out through the top, thus bursting apart the four terminal **lip-cells.** The central channel of the neck is filled by the **neck canal cells** which are not separated from one another by cross walls. Below them is the **ventral canal cell** and finally, in the venter at the base, the **oosphere.**

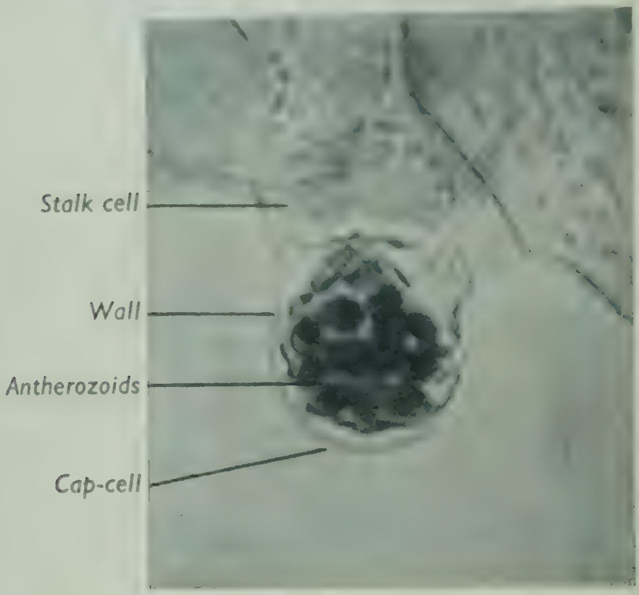
Stalk cell
Wall
Antherozoids
Cap-cell

FIG. 28.—*Dryopteris filix-mas.* Vertical section of the antheridium.

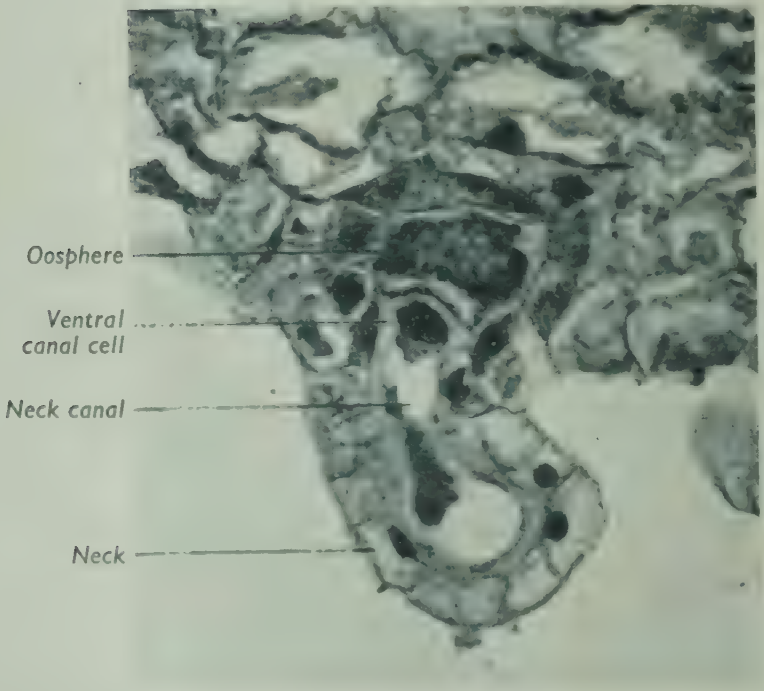
Oosphere
Ventral canal cell
Neck canal
Neck

FIG. 29.—*Dryopteris filix-mas.* Vertical section of the archegonium.

The venter is sunk in the tissues of the cushion and cannot be clearly separated from it. It encloses the ventral canal cell and the oosphere which lies at the bottom. In the region of the ventral canal cell the canal enlarges, and the upper wall of the ventral canal cell forms a dome-like membrane across the cavity.

If the section has been taken appropriately it will be seen that as the archegonium matures the neck bends over until the upper end comes to lie almost parallel to and close against the surface of the prothallus.

Young Sporophyte.—Mount a young plant still attached to the prothallus on a slide and examine under a hand lens.

At a very early stage the prothallus shows no morphological change, and the young embryo appears only as a green leaf which grows up between the two lobes of the prothallus from its under side. The first leaf is usually bilobed, and it is only through a series of intermediate stages that the pinnate form of adult leaf is established.

In a more mature young sporophyte the primary root makes its appearance at one end and the young shoot apex at the other, while the tissues of the prothallus lose their bright green colour, turn brown, and gradually disorganize. Up to that time part of the nourishment required by the sporophyte has been derived from the prothallus through the **foot,** a structure arising from the side of the embryo, in the region of the original archegonium and embedded in the prothallial tissues like the foot of a moss sporogonium.

It will be observed that as a rule only one sporophyte develops on each prothallus, although several archegonia may be fertilized. The unsuccessful embryos disappear.

2. ***Pteridium.*** *External Morphology.*—To see the whole structure of *Pteridium aquilinum*, the Bracken Fern, it is necessary to dig deeply, for the rhizome generally lies at a considerable depth.

When a good length of the **rhizome** has been uncovered it will be seen to consist of a blackish-brown, horizontally growing stem, which bears at intervals long upright **petioles** which are, at their lower ends, the same colour as the rhizome, but as they pass above the ground become thinner and green in colour. From these petioles branches are given off pinnately on which the laminae are developed, producing the characteristic Bracken foliage.

Compared with *Dryopteris*, the petioles are more widely separated from one another, with the result that the anatomical study of the rhizome can be made without the difficulty of allowing for the tissue alteration in the leaf gap. Adventitious roots are given off by the rhizome, which are thin and wiry and are similar in appearance to those found in *Dryopteris*, except that in *Pteridium* they arise at any point on the rhizome. The growing apex of the bracken is not covered by the overlapping leaves as in *Dryopteris*, but is mainly protected by a single petiole developed immediately below it.

Ramenta are almost entirely absent from the rhizome and petiole of *Pteridium*, but their place is taken by brown hairs which cover the young frond but drop off as it develops.

As the rhizome continues to grow the older parts at the opposite end die away, together with the petioles of the leaves of previous years, but the process of decay is slow, so that rhizomes several yards long may be found crossing each other and binding the soil together. It should be noted that the fronds of Bracken all die down in the autumn and that new ones are developed during the following spring.

> *Transverse Section of the Rhizome.*—Cut a transverse section through the rhizome, stain in Aniline chloride and mount in Glycerine. Examine the preparation first under a hand lens.

There is a thick band of sclerotic cells, surrounding the outside of the rhizome, made up of heavily thickened cells which are stained bright yellow. This is followed by a wide zone of thin-walled cells filled with starch grains, the parenchymatous **ground tissues,** in which are arranged a ring of **meristeles,** some of which are roughly circular in outline, while others are considerably elongated. Two large bands of **sclerenchyma** lie towards the centre of the section. These serve to orientate the section, for the curved band lies on the upper side of the rhizome and the flat plate on the lower. Internal to these two bands, which form a rough semicircle, is a further group of meristeles, which may consist of one or two steles only or may be split up into a number of smaller segments. The degree to which the various meristeles are segmented depends upon the proximity of the leaf trace, and the most uniform conditions are found in sections cut well away from foliar interference. The form of the stele is an elongated network of meristeles, as in *Dryopteris*, but in this case the gaps are not all leaf-gaps, but simple perforations.

Under the microscope the outer sclerotic tissue will be found to be most thickened on the outside, becoming less heavily thickened inwards towards the cortical parenchyma, where only slight lignification of the walls occurs. The ground parenchyma is composed of hexagonal cells with well-marked nuclei, and scattered in this tissue may be found isolated groups of sclerotic cells.

The essential structure of all the meristeles is the same. The stele is separated from the ground tissue by an **endodermis** composed of cells whose inner tangential and radial walls are slightly thickened. The **pericycle** consists of small oval cells forming a ring, one cell wide, immediately inside the endodermis. The **phloem** lies in contact with the pericycle, forming a narrow band of tissue, continuous except at the ends where the **protoxylem** is situated. The xylem has two protoxylem groups which lie towards either end of a band composed of **metaxylem.** As the protoxylems are surrounded by metaxylem elements the structure is said to be **mesarch.** Between the xylem and phloem there may be a small amount of xylem parenchyma.

Longitudinal Section of the Rhizome.—Cut a radial longitudinal section of the rhizome, stain in Aniline chloride and mount in Glycerine.

The outermost tissues consist of a layer of elongated cells with dark-coloured walls. These cells are similar to those composing the sclerenchyma bands, except that their walls are not so thick.

The **cortex** is made up of rectangular parenchyma cells, densely filled with irregular starch grains. The **sclerenchyma bands** are deeply stained and are made up of elongated, thick-walled cells, with more or less oblique end walls and numerous minute pits.

Several meristeles may be cut in a single section and they should be compared with one another. The **endodermis** appears deeply stained because of the thickening on its radial walls. The **pericycle,** on the other hand, is colourless and thin-walled. The **phloem** shows very narrow elements externally and broader elements towards the centre. These are either sieve tubes or phloem parenchyma. On the sieve tubes it may be possible to see the numerous lateral **sieve fields.**

If the section passes through a **protoxylem** it will be seen to consist of spiral elements surrounded by xylem parenchyma. The elements of the **metaxylem** have scalariform pits arranged in from one to four rows according to the width of the element.

Transverse Section of the Petiole.—Cut a transverse section through a fairly young petiole, stain in Aniline chloride and mount in Glycerine. Examine in the first instance with a hand lens.

Compared with *Dryopteris* the arrangement of the tissues is far more complex. There are considerably more meristeles, which appear at first sight to be arranged in no definite pattern. It can be shown, however, that if the individual meristeles are carefully analysed with regard to the orientation of their tissues, they will be found to be arranged in a highly convoluted horse-shoe similar to, if somewhat more complicated than, that in *Dryopteris.*

The detailed structure of the ground tissue and of the separate meristeles differs in no marked way from that described in *Dryopteris*, and need not be repeated here. It will be found that as most of the elements are larger it is easier to make out the details of the structure in these sections than in the Male Fern.

If permanent stained preparations of either the petiole or the rhizome are attempted, and they are desirable structures to experiment with, it will be found that very frequently there is present in the cells a substance which precipitates Haematoxylin. This can be removed with acid alcohol, but it is better to use aniline dyes for staining Fern material, for they do not precipitate.

Transverse Section of the Root.—See *Dryopteris filix-mas*, page 134. The roots of ferns show a very remarkable similarity not only among present-day types but also among fossil forms, and it is unnecessary for us to consider the root of *Pteridium* separately.

Transverse Section of the Leaf.[1]—Cut a transverse section through a young leaf, stain in Aniline chloride and mount in water.

The **lamina** appears quite thin in comparison with the **midrib,** which on the lower surface is enlarged as an oval mass of thickened cells. On the upper surface it is covered by a similar mass of tissue, but here, instead of being oval in outline, the tissue is raised into two ribs with a shallow cavity between.

The structure of the midrib itself agrees essentially with that already described in *Dryopteris.* The lamina is bounded above and below by large oval **epidermal cells,** while the cells of the **mesophyll** are rounded, with large **intercellular spaces,** and are densely packed with discoid chloroplasts.

At the margin of the lamina the upper epidermis is extended round to form a **false indusium** below the leaf, while a **placenta** is formed from tissue developed from the true edge of the leaf. From this placenta numerous stalked sporangia arise. These sporangia are essentially the same in structure as those of *Dryopteris*, but as a rule there is a greater variation in age of the sporangia seen in a section of *Pteris*, and for this reason it is preferable to *Dryopteris* for a study of developmental stages. In a section it is possible to find young sporangia in which the archesporial tissue can be recognized while close by are sporangia with mature spores. It must be realised that in *Pteris* the whole margin of the leaf bears sporangia, and that they are not restricted to special areas as in *Dryopteris.*

If *Pteridium aquilinum* is used in place of *Pteris serrulata* a membrane will be seen arising from the inner side of the placenta, between this and the lower surface of the leaf. This tissue is regarded as a **true indusium,** and its presence serves to separate the Bracken from the genus *Pteris.*

3. ***Hymenophyllum tunbridgense.*** *External Morphology.*—Examine fresh or herbarium material of a fertile specimen.

The plant consists of a thin, wiry **rhizome**, from which the **fronds** arise at short intervals, interspersed with very fine, hair-like **roots**. **Pinnae** are given off alternately on each side of the **rachis,** each pinna branching dichotomously several times. The lamina of the pinnae is only one cell thick and is a translucent green ; hence the name " Filmy Fern." A single midrib without side veins traverses each pinna segment. The exact form of the frond is very variable. The size is also variable but is usually less than three inches in length.

The **sori** are formed at the base of the pinnae, each representing one arm of the first dichotomy. Each sorus is pouch-shaped and is enclosed between two, partly united **indusia,** which arise from the upper and lower sides of the pinna respectively. Generally only one sorus is present on each pinna, but this is variable.

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[1] The description given refers to *Pteris serrulata*, the Ribbon Fern, common in greenhouses, which may be advantageously substituted for *Pteridium* for leaf structure.

Transverse Section of the Rhizome.—Cut a transverse section of the rhizome, stain in Aniline chloride and mount in Glycerine.

The **cortex** is sclerotic towards the outside and thinner walled inwards. In the centre is a well-marked **stele,** with a dark coloured **endodermis** and a **pericycle** which is one to several cells in width. The **xylem** forms a solid, central mass, with the **protoxylem** in the middle. The **phloem** forms a narrow zone surrounding the xylem. A certain amount of parenchyma is scattered among the xylem cells, and may form a distinct zone around the protoxylem. The **leaf traces** are simple collateral strands.

Transverse Section of the Petiole.—Cut a transverse section of the middle portion of the petiole, below the pinnae ; mount in Glycerine.

The **cortex** is highly sclerotic, almost throughout. The **stele** is surrounded by a well-marked **endodermis** and **pericycle**. The **xylem** forms a somewhat curved arc in the upper portion of the petiole, but towards the base it forms only a rounded mass, surrounded by **phloem.**

Reproduction : Sorus and Sporangia.—Cut a fairly thick section longitudinally through a sorus and mount in Glycerine.

The **sorus** arises directly from the edge of the pinna, and the lamina is continued directly upwards into the **indusium,** which is two-lipped. Between the lips of the indusium there arises a short stalk, the **receptacle**, on which are formed the **sporangia.** The latter arise in basipetal succession, the oldest being, therefore, those at the top. The receptacle elongates from its base as the lower sporangia mature. The sporangia are short-stalked, and each shows a very prominent, **oblique annulus,** with bright brown cell walls and larger cells.

4. ***Osmunda regalis.*** *External Morphology.*—Examine a fertile plant.

The stem of *Osmunda* is a short thick, subterranean stock, which is thickly surrounded by the stumps of old leaf petioles. Each year a fresh group of leaves is formed, in spiral succession, the first leaves to appear being fertile, *i.e.*, bearing sporangia, while the later leaves are sterile. The latter arch outwards, but the fertile leaves remain erect. The stems branch only in old plants. They fork dichotomously and may build up, in time, large clumps. The leaves may be up to 6 ft. long and 1 to 2 ft. broad. They are bipinnate and the **pinnules** are broad, obtuse, and measure up to 6 cm. long by 1 cm. broad. Each is attached to the rachis at one point only and has a short midrib with pinnate side veins. The margins of the pinnules are entire at the apex but crenate towards the base, the lowest segments being sometimes separated from the rest of the lamina, as auricles.

In the fertile fronds the uppermost five or six pairs of pinnae bear the **sporangia.** These pinnae have no laminae and the midribs of the pinnules bear sporangia in a series of dense clusters which entirely hide the midrib. When young the sporangia are bluish-green, but become dark-brown when old. The lower pinnae are like those on the sterile leaves. It is noteworthy that the insertion of the pinnules on the rachis is almost horizontal, rather than vertical as in most Ferns, and that they assume the vertical position by the twisting of the base of the pinnule. This may be a relic of an earlier three-dimensional branching of the frond.

> *Transverse Section of the Petiole.*—Cut transverse sections through the middle part of a petiole, below the lowest pinnae; stain in Aniline chloride and mount in Glycerine.

The adaxial side of the petiole is marked by a shallow groove. The vascular tissue take the form of a horse-shoe, the open side directed adaxially, with the ends incurled. Under the microscope this tissue will be seen to consist of a median band of **xylem,** formed of rather uniform elements, with a number of **protoxylem** groups of small cells, on the concave side of the band. On both sides of the xylem is a band of **phloem,** which is continuous round the ends of the horseshoe, that on the inner side being somewhat interrupted by the protoxylems. A zone of selerotic cells, possibly pericycle, encloses the whole vascular strand, but no true endodermis is distinguishable.

> *Reproduction: Section through a Sporangiate Pinnule.*—Cut a fairly thick transverse section so as to retain a number of attached sporangia and mount in Glycerine.

The pinnule is somewhat oval in section, with a single vascular bundle, the **sporangia** arising chiefly from the two ends of the oval. Each sporangium has a stout, multicellular stalk and has a wall two or three cells thick, the outer layer containing dense contents, which turn brown at maturity. The **spores** are small, smooth-walled and each is marked on one side with a tri-radiate slit, by which the spore dehisces.

The sporangium itself dehisces by an apical slit, which terminates at a group of thick walled cells at the side of the apex. This group may represent a rudimentary annulus. The line of dehiscence is marked by specially small cells in the outer wall.

> 5. ***Botrychium lunaria.*** *External Morphology.*—Examine a fertile plant.

At the base is a short, subterranean **stem,** from which arise a number of spreading **adventitious roots.** From this stem arises a thick, green **petiole,** the base of which is sheathed in the membranous remains of leaves of previous years. A few inches above the ground level the petiole divides into a **fertile** and a **sterile frond**. The latter is simply **pinnate,** each fan-shaped **pinna** overlapping the one next above it. The margins of the pinnae are irregularly crenate and they are attached to the **rachis** by short, substantial stalks. The fertile frond is **bipinnate** and each **pinnule** bears two **marginal rows** of large **sporangia.**

Transverse Section of the Stem.—Cut a transverse section of the rhizomatous underground stem, stain in Aniline chloride and mount in Glycerine.

The ground tissue is thin-walled and rich in starch grains. In the centre is a small irregular ring of three to four **meristeles,** each of which is **collateral** in structure, with a scanty **xylem** on the inner side and relatively well-developed **phloem.** No endodermis is distinguishable. In the cortex may be seen small collateral **root traces** and sometimes the broader, curved strands which supply the base of the petiole.

Transverse Section of the Petiole.—Cut a transverse section at about the middle of the petiole, below the point of division; stain in Aniline chloride and mount in Glycerine.

The anatomy of this portion of the petiole is simple. There is an abundant **ground tissue,** in the middle of which lies a curved band of **vascular tissue,** which is generally divided into two portions. The vascular band is collateral, the **xylem** forming a broad zone at the concave side, with the **phloem** on the convex side. The ends of the vascular band are slightly incurved. The pinna traces, if present, will be seen coming off from the convex side, close to, but not at, the incurved ends.

Reproduction : Sporangia.—Cut sections across a fertile pinnule, in such a way as to cut through a pair of sporangia. Sections need not be specially thin. Mount in Glycerine.

Sections somewhat resemble those through an anther with two pollen sacs. Between the sporangia lies the pinnule itself, containing a single vascular bundle which gives off two small branches, right and left, at the level of attachment of the sporangia. These branches splay out and end blindly at the base of the sporangia. The sporangia are practically sessile on the pinnule. Each sporangium has a substantial wall, several cells thick, of which the cells of the outer layer are large, square, and cuticularized all round. The inner wall layers are thin-walled and flattened.

If the sporangia are immature, the **tapetum** may be seen in the form of plamodial protoplasm which penetrates throughout the sporangial cavity among the developing **spores.** The latter are small and thick-walled.

6. ***Azolla.*** *Vegetative Morphology.*—There are two British species, *Azolla caroliniana* and *Azolla filiculoides*, which occur floating on the surface of water in ponds and ditches, sometimes covering the entire surface with a dull green, feathery layer of fronds. In autumn the plant often turns bright crimson. Although widely spread in the southern part of the country, it is not a native plant, having been first noted in 1911.

Our description is taken from *A. filiculoides*, which, however, differs from the other species only in minor details.

Place some fronds in water in a flat dish and examine with a hand lens.

Viewed from the upper surface each shoot is roughly triangular in outline, about one inch long, bipinnately branched, with the branches alternating on two sides of the main stem, so that the shoot is more or less flat. Each branch is covered with small closely imbricated leaves, each with a membranous margin. On the older stem the leaves are more widely separated. The leaves alternate in two rows on the upper side of the stems.

On turning the shoot over so as to view the lower surface, we see the submerged lobes of the leaves, which are colourless and membranous throughout. They are probably absorptive. Numerous unbranched adventitious roots arise from the upper side of the stem and from the basal part of each of the branches. The plants easily fragment and multiply vegetatively by this means.

In fertile plants, male and female **sporocarps,** or enclosed sori, are usually both present. They are borne on the submerged lobes of the lowest leaf of each branch. The fertile lobe is normally divided into two, with a sporocarp borne terminally on each lobe. The paired sporocarps may either be both male, both female, or one of each. The membranous margin of the upper lobe of the leaf is here greatly expanded and covers the sporocarp.

Male sporocarps are rounded, almost spherical, with a pointed apex, and about 1·5 mm. in diameter. The pale yellow microsporangia can be seen through the covering membrane. The female sori are much smaller, narrower and more pointed, measuring about 0·25 mm. broad and 0·75 mm. long. Each has a dark brown apex.

Microscopical Structure.—Mount one or two small portions, *i.e.*, short branches, in Lactophenol-Cotton Blue and tease out with needles. Examine under a cover-glass.

The stem shows a small central stele, consisting of a few very long spiral elements, with a scanty phloem and surrounded by a large-celled cortex, without any specialised epidermis. Root initials arise endogenously from the lower side of the stem, close to the points of origin of the side branches. Each root contains a single central strand and has a well-marked root cap of radial cells. The lateral branches arise alternately on each side of the stem, above every third leaf, but they are not axillary.

The leaves are duplex, as remarked above. Each receives a single trace bundle which forks at the point of division of the leaf, one branch going to each leaf lobe. That in the lower submerged lobe takes a straight median course and ends about two-thirds of the way to the apex. The bundle in the upper aerial lobe makes a wide curve around the margin of the principal internal cavity of the leaf, showing an outline like a fish-hook, and ending near the leaf base in a group of short tracheids. The bundles in both lobes are unbranched, but that in the upper lobe shows a short appendage at the top of its curve.

The **aerial lobe** of the leaf stands out almost at right angles to the stem. It is thickest at the base, and the outer edge of the thickened portion is sharply marked off from the marginal membrane, which is devoid of active chloroplasts and is only one cell thick, though three or four cells broad. The epidermis is composed of elongated cells with fairly straight walls. On the abaxial (lower) side it is thickly set with conical, unicellular, papillate hairs, which produce a velvety, unwettable surface. These are practically absent from the upper surface.

Both surfaces bear peculiar **stomata,** but they are more numerous on the upper surface. Each stoma is formed from two cells, but the elongated pore lies at right angles to the wall between them. This wall later disintegrates, leaving the pore surrounded by a single ring-like cell, with two nuclei. The structure recalls the stomata on the capsule of *Funaria* and some other mosses. All the epidermal cells, including the guard cells, contain chloroplasts, and there is no evidence of any closing mechanism. There are no stomata on the marginal membrane.

On the upper leaf surface, near the base, there is an opening which may be a modified stoma, guarded by papillate hairs, communicating with a large **internal cavity.** This cavity occupies all the upper portion of the mesophyll, interior to the crook of the vascular bundle. It is lined by a layer of cells with wavy outlines like those of a typical epidermis and contains numerous filaments of the Blue-green Alga, *Anabaena azollae,* which stain brightly with Cotton Blue. Certain large glandular hairs which grow into the cavity from the surrounding wall suggest an absorptive function, and it is possible that the commensal Alga assists in the nitrogenous nutrition of the *Azolla,* which is seldom found without it.

Beyond the vascular crook and also within it, but below the *Anabaena* cavity, the mesophyll tissue consists of radial strands of chlorenchyma cells, separated by wide intercellular spaces, often saccate in appearance. The *Anabaena* cavity may simply be a specially large mesophyll cavity of the same type.

The **submerged lobe** of the leaf is similarly constructed, but on a reduced scale. The vascular bundle is straight and median. There is no *Anabaena* cavity and no pore on the adaxial surface. The thickened mesophyllar portion is smaller and the membranous margin is much wider. The mesophyll tissue is very scanty, with wide, saccate spaces, but there is no *Anabaena* infection as there is no portal of entry.

> *Reproduction: The Microsporocarp.*—Tease out a microsporocarp in a drop of Glycerine on a slide, cover, and crush the sporangia by tapping vigorously on the cover-glass.

The microsporocarp is surrounded by a delicate transparent membrane, forming a sac, at the base of which is a short **columella.** From this there arise, in basipetal succession, numerous sporangia on thin stalks of varying length, so that the sporocarp is closely filled.

The sporangia are spherical and have no annulus. In young sporangia the minute brownish microspores can easily be seen, but with advancing maturity the **periplasm** becomes more abundant and granular and obscures the spores. The most obvious contents of the sporangium at this stage are the outlines of the vacuoles in the periplasm. These vacuoles enlarge and fuse until only from five to eight remain, the spores being distributed among them. Each vacuole forms one **massula,** surrounded by a layer of the remains of the periplasm within which it has developed.

The massula consists of a protoplasmic honeycomb, containing up to a dozen microspores. When the sporangia are ruptured by pressure the massulae should be extruded. The walls of its superficial vesicles are at many points elongated radially outwards into tapering, hair-like structures with anchor-shaped heads and recurved barbs. These are the **glochidia,** by means of which the massula may become attached to the pitted wall of a megaspore.

It is difficult to realise on examination of a massula that one is really looking at a highly specialised vacuole and that the glochidia are not cellular but purely protoplasmic structures. They are among the most remarkable specializations of protoplasm in Nature.

> *Reproduction: The Megasporocarp.*—Pick off megasporocarps and mount two or three in Glycerine. Mount others separately and tease them out under a lens to reveal their contents.

The intact megasporocarp is lemon-shaped and stands on a short dark-coloured stalk. The sporocarp wall closely covers the single contained megasporangium, and at maturity the sporangium wall is usually indistinguishable. The basal part of the sporocarp wall is transparent, and through it may be seen the pitted wall of the megaspore, which fills all the lower half of the sporangium. The upper half of the sporocarp wall is brown and less transparent.

Examine the crushed preparation. The contents should be extruded, and the **perispore** of the megaspore will reveal a pattern of ragged-edged craters all over its surface. On top the megaspore is more or less flattened into three faces, separated by ridges which are the lines of future dehiscence.

Above the megaspore the upper part of the megasporangium is occupied by three oval bodies, each, like a massula, the product of a periplasmic vacuole and consisting of the same kind of protoplasmic honeycomb, but without glochidia and containing only the abortive megaspores. They are, in fact, sister vacuoles to that in which the fertile megaspore develops. These three bodies constitute the **floats.** They adhere to the central point on the upper surface of the megaspore, which is prolonged into a short beak, and they are shed along with the spore and provide for its buoyancy.

If the upper part of the sporocarp wall has been pulled off in making the preparation, the periplasm investing the floats will be seen as a layer of fine, tangled filaments, recalling the capillitium of a myxomycete sporangium.

Section 8. Equisetales

Equisetum. *External Morphology.*—Examine a complete plant of *Equisetum arvense*, the Field Horsetail, which has been dug up carefully, washed free of soil and dried.

The plant consists of an underground **rhizome** from which numerous **aerial branches** arise. The rhizome is divisible into nodes and internodes, at each node there being a sheathing whorl of **leaf scales** forming a ring around the stem. These leaf scales occur both on the rhizome and on the aerial stems, though in the latter they are green with brown tips, while in the former they are all brown. Arising at the nodes of the rhizome are numerous **adventitious roots** which are fairly short and are irregularly branched. Small oval **root-tubers** are also developed here and there from the nodes. The rhizome may be branched, each branch arising also at a node.

The character of the aerial stem depends upon whether it is a fertile or a sterile one. The **fertile stems** appear in March and are yellowish-brown in colour, being entirely devoid of chlorophyll, and are destined only to bear terminal fertile heads of sporangia. They die soon after the spores have been shed.

Later the **sterile stems** appear. They are green in colour and branched. The branches arise in whorls from successive nodes, in the axil between the stem and the leaf sheaths, and are equal in number to the leaves. They break through the base of the leaf sheath so that they appear to come from below it.

The surface of the stem is deeply ridged lengthwise, each rib being in line with the leaf immediately below it and alternating with a rib of the next internode. Thus leaves and ribs alternate in successive internodes.

It will be noted that the leaves are extremely small and consequently the function of photosynthesis is almost entirely performed by the aerial stems and the branches. The branches are jointed like the main stems, with a minute sheath of leaf-scales at each node.

Transverse Section of the Aerial Stem.—Cut two sections of the aerial stem, one through a node and the other through the internode; stain in Aniline chloride and mount in Glycerine.

The anatomy of the stem is complicated by the fact that it serves the function of an assimilatory tissue in addition to its normal purposes. This modification is seen especially in the cortex, where there are alternating zones of **sclerenchyma** and of parenchymatous **assimilatory tissue.** The sclerenchyma is composed of thick-walled cells and occupies the tips of the ridges, while the assimilatory tissue is thin-walled and lies in the grooves between the ridges. In this way the assimilatory cells are protected both from desiccation and from damage. The **stomata** lie in the bottom of the grooves.

The inner part of the cortex consists of parenchymatous cells with large

intercellular cavities alternating with the vascular bundles. This regular arrangement of internal cavities is a characteristic of the group. These cortical cavities are called the **vallecular canals,** as they are opposite the grooves.

There is a well-marked **endodermis** surrounding the ring of vascular bundles and composed of cells, oval in section, whose radial walls are slightly thickened. Inside the endodermis lies the **pericycle,** composed of cells very like those of the endodermis. These two layers enclose a single ring of vascular bundles, so that the structure is monostelic, not meristelic as in the Ferns.

The **vascular bundles** are arranged in a ring. Each consists of two small arms of **metaxylem** which are inclined at an angle, but the apex of the angle, which originally consisted of the **protoxylem,** is represented by a cavity, the **carinal canal,** which is formed by the secondary breakdown of the protoxylem elements. Usually the remains of one or two protoxylem elements may be seen attached to the walls of the canal. Between the two metaxylem arms lies the **phloem,** which is made up of sieve tubes and parenchyma. The ring of vascular bundles encloses a parenchymatous **pith,** which in the internodes is usually hollow.

If the section has been cut at a node the arrangement of the parts becomes more complex. The xylem increases greatly in amount and obliterates the carinal canals, while the xylems of adjacent bundles contribute two strands which pass out through the cortex to provide the bundles for the branches, forming almost horizontally running strands. Immediately below, and alternating with these traces, is another series of trace bundles, smaller in size and also derived from two vascular bundles, which pass out to the scale leaves. It will be noticed that since each trace derives part of its tissue from the two adjacent vascular bundles, the one half of each stem-bundle supplies a branch while the other half contributes to a leaf trace.

The second point to notice in the nodal section is the solid pith. In other respects all sections resemble one another in essential points.

If a section be cut across one of the small branches it will be seen that the same general structure persists, but that the tissue arrangement is somewhat simplified. There is a single layer of sclerotic cells on the outside, interrupted here and there in the grooves by stomata, beneath which are the assimilatory cortical cells. There is a small number, about four, of large cortical air spaces. The endodermis is sharply differentiated and the tangential walls appear thickened. The pericycle is made up of small, polygonal cells. There are usually four vascular bundles, each with a carinal canal at its inner edge. Each bundle is made up of a few isolated xylem elements and a mixed tissue of phloem and parenchyma. The stelar tissue is embedded in a large celled parenchymatous pith with no central cavity in it.

Transverse Section of the Rhizome.—Cut a transverse section through the rhizome, stain in Aniline chloride and mount in Glycerine.

The anatomy of the rhizome is in many respects similar to that of the aerial stem. There is a single-layered **epidermis** composed of brick-shaped cells, inside which are one or two layers of small-celled **sclerenchyma.** This is followed by a parenchymatous **cortex** which towards the centre becomes lacunar, with large spaces separated by rows of thin-walled cells. The **endodermis** is external to the vascular bundles. It is not thickened all over, but a **Casparian band** is clearly visible on the radial walls of the cells. At the nodes a partial endodermis occurs on the inner side of each vascular bundle. The pericycle is not markedly differentiated.

The **vascular bundles** form a ring. Each bundle contains two groups of **metaxylem** elements, separated by the **carinal canal,** within which may usually be seen one or two lignified elements which represent the remains of the **protoxylem.** Towards the outside and bounding the canal lies the **phloem,** which is made up of a small number of sieve tubes and a large amount of parenchyma. The bundles are embedded in a parenchymatous **pith** which becomes broken down towards the centre forming a large cavity.

Starch grains occur in the cells of the inner cortex and also in the parenchyma surrounding the vascular bundles.

Longitudinal Section of the Aerial Stem.—Cut a tangential longitudinal section of the stem through a node (the plane of the section should just touch the stele), stain in Aniline chloride and mount in Glycerine.

The purpose of this section is to clarify the observations made in the transverse section of the stem through the node. The vascular bundles are the most noticeable feature. They will be seen as a series of parallel strands, each consisting of a thin central strand of protoxylem with ring thickening, flanked on each side by a group of metaxylem elements, between which may be glimpsed phloem cells, with lateral sieve plates. If the section has been carefully made and is truly tangential to the stele, it will be possible to trace the course of the bundles through the node. It will be seen that immediately below the node the bundles divide and diverge so that each bundle of the internode above is made up of halves of two bundles from the internode below, joined together. Immediately at the bottom and top of each arch of xylem so formed will be found a little group of xylem cells cut across at right angles. These are in two whorls, one slightly above the other, and alternating with one another. The lower and smaller whorl is composed of the traces passing out to the scale leaves, the others, arising from the upper side of the arches, are larger and pass to the branches.

Few other details of interest will be seen in the section, for most of the other tissues are obscured by the vascular bundles, but in the cortex may be seen either the longitudinally running cells of sclerenchyma or the assimilatory tissue with its associated stomata.

Transverse Section of the Root.—Cut a transverse section of the root, stain in Aniline chloride and mount in Glycerine.

The roots are very slender and arise adventitiously from the rhizome. In a transverse section we may recognize a thin-walled **exodermis** on the outside, followed by a thick **hypodermis,** and probably one layer of cortical parenchyma. Both outer layers are covered with a black deposit. The **endodermis** is composed of cells which are oval in section, with thickened radial walls, and an inner layer of smaller cells without thickening which take the place of the **pericycle.** There may be two or three **protoxylem** groups, with one or two central **metaxylem** cells, and alternating with the protoxylems are small groups of very small **phloem** cells.

Except in having a two-layered endodermis, the inner layer of which replaces the pericycle, a point which has been proved by a study of its development, the root of *Equisetum* does not differ in any important way from that of a higher plant.

> *Morphology of the Strobilus.*—Examine a mature cone or **strobilus** under a dissecting microscope.

It is made up of alternating whorls of peltate scales. These scales consist of a flat hexagonal plate attached to the main axis by a stalk at right angles to the plate. The whole structure is generally referred to as a **sporangiophore.** There are about twenty of these sporangiophores in each whorl.

Each sporangiophore bears on the inner surface of the head about nine elongated sac-like **sporangia** which surround the stalk and completely fill the space between the main axis and the head.

At the base of the cone is a whorl of scale leaves of a rather rudimentary type which is collectively spoken of as the **annulus.**

In a longitudinal section made through a whole young cone certain additional structures can be made out. The vascular anatomy is somewhat similar to that in the sterile shoots, a branch of a vascular bundle passing along the stalk of each sporangiophore and dividing up in the peltate head to supply each of the sporangia.

The sporangia are elongated sacs with walls which are one cell thick when mature, though at an earlier stage the wall appears to consist of two layers, the sporangium wall proper and the **tapetum.** The contents of the sporangium will depend upon the age of the cone. If young it may consist of a solid tissue, the **archesporium,** in the cells of which meiotic nuclear division occurs and may sometimes be seen. If mature the sac will contain separate **spores.**

Mount some spores from an unopened sporangium, either fresh or preserved, on a slide, without liquid or a cover-glass, and allow them to dry while observing them under a microscope. The damp spore will be seen to be enclosed in an **epispore,** which forms the outermost investment of the spore. This is additional to the normal wall of the spore which consists of three layers. This epispore splits along spiral lines into two bands with broad ends, and these spirals expand as the spore dries. When the spore, therefore, has dried sufficiently, these bands will be seen as two large spirals, one on each side of the spore and easily detached from it. When fully expanded the epispore bands flatten out and form a cross, with the spore at the point of intersection. If a drop of water is now introduced the appendages curl up again, but not necessarily with the spore inside. We conclude, therefore, that the epispore is hygroscopic and that the action of the spiral bands is both to liberate and disseminate the spores.

Prothallus.—The male and female sex organs are usually produced on the same prothallus. In the youngest stages only antheridia are formed, so that small prothalli are entirely male, while archegonia are only found on the larger and older specimens. Prothalli may be obtained easily by sowing fresh spores on sand and keeping them moist.

The prothallus consists of a multicellular central region which gives off numerous flattened branches composed of elongated but very irregular cells.

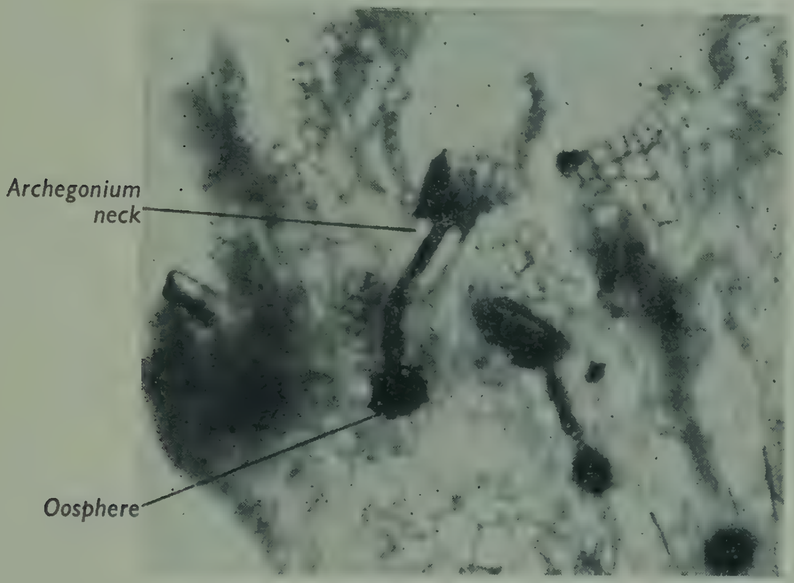

FIG. 30.—*Equisetum arvense.* Prothallus with archegonia.

These branches may themselves again branch until they terminate irregularly in uniseriate filaments. The central tissue is more regular and is several cells thick, and from these cells **rhizoids** arise, which are long, brown, unbranched structures.

Towards the upper end of the central tissue, that is, towards the end where the branches arise and opposite that at which the rhizoids are developed, numerous **archegonia** may be found. The archegonium is partly immersed in the prothallus tissue (Fig. 30), and consists of a neck and a venter which is composed of the same arrangement of cells as in the archegonium of the Male Fern. It will be noticed in this connection that there is a far closer similarity between the archegonia of *Equisetum* and *Dryopteris* than between *Equisetum* and either *Pellia* or *Marchantia* on the one hand, where the neck is so considerably elongated, or *Selaginella* on the other, where the neck is even shorter.

The prothallus bearing **antheridia** is less complexly branched than that bearing archegonia, and at the time when the mature antheridia are produced is considerably smaller (Fig. 31). The antheridia may be found either at the base of or upon the branches of the prothallus. The structure of the antheridium is essentially similar to that already described in *Dryopteris*, page 137, although it is considerably larger.

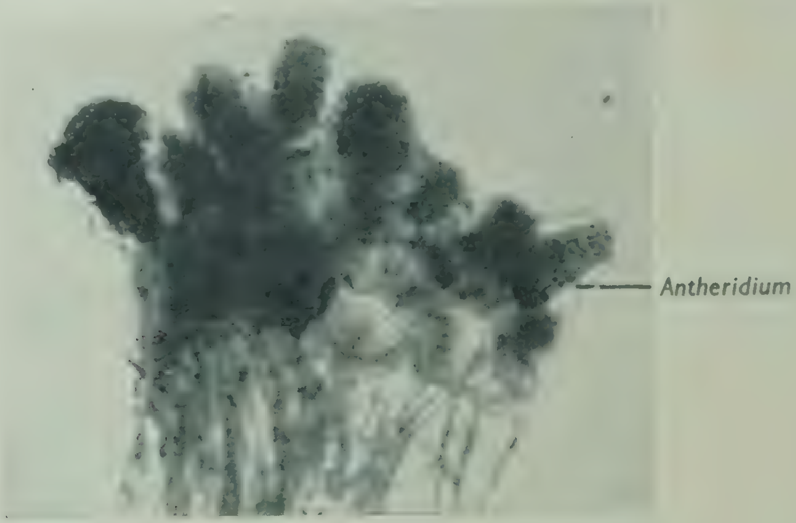

FIG. 31.—*Equisetum arvense.* Prothallus with antheridia.

Section 9. Lycopodiales

1. ***Lycopodium clavatum.*** *External Structure.*—The plant is prostrate and densely clothed with small, linear, lanceolate, incurved leaves, spirally arranged.

The stem is rigid and much branched, varying in length from one to as much as three feet. Adventitious roots are given off along the stem and may grow for some time as aerial organs before entering the soil where they become much branched.

The **strobili** are borne on stiff, erect peduncles, either solitary or in pairs, the peduncle being provided with minute scale leaves. The strobili consist of closely packed **sporophylls,** cylindrical and pointed, and are considerably longer than broad.

The species is not uncommon on moors and heaths, particularly in more mountainous parts of the west country, from Scotland to Cornwall.

Transverse Section of the Stem.—Cut a transverse section of the stem, stain in Aniline chloride and mount in Glycerine.

The section will show a number of leaf bases attached around the outside, producing a very irregular outline, the tissue formed thereby being strongly lignified. Internal to this there are a number of rows of parenchymatous

cortex cells which are followed on the inside by a zone of sclerenchyma, the **inner cortex,** the cells of which become increasingly thickened as the centre is approached.

The central **stele** is bounded by a **pericycle** which in section appears very similar to an endodermis. The vascular tissue is made up of about four parallel **xylem bands,** those on the outside being branched. The **protoxylem** lies at the two edges of each band, but in the branched bands they are at the edges of each branch. In this way a ring of exarch protoxylems, about a dozen in number, come to lie around the periphery of the stele. The spaces between these bands is filled principally by **phloem.**

In the inner cortex may be seen the leaf traces, formed in a spiral, each successive bundle being farther away from the centre. While those nearest the centre are cut transversely, those farther out are finally cut longitudinally, and it may be seen that where the traces lie in the thin-walled part of the cortex they are surrounded by thick-walled sclerenchyma. It will also be found that these cortical bundles are produced in connection with each of the leaf bases, observable around the outside of the section, showing that they are destined to become the traces of the leaves cut in the section. They originate from protoxylems at the edges of the xylem bands.

> *Transverse Section of the Root.*—Transverse sections cut through the aerial root are stained in Aniline chloride and mounted in Glycerine.

The whole of the outer tissue is heavily sclerotic. The **cortex** consists of an irregular outer layer of large cells, followed by successive layers of small strongly thickened cells, and bounded on the inside by an **endodermis** of slightly larger cells but still strongly thickened.

The stelar anatomy is entirely comparable with that of the stem, with a small number of **xylem bands,** which may either stretch across the stele or may split up or branch. The **protoxylem** lies to the outside in each case, so that a ring of protoxylem groups is arranged around the periphery. The **phloem** is composed of sieve tubes and lies in between the xylem bands.

This similarity of character between the root and the stem of *Lycopodium* is one of the interesting features of the plant.

> *Longitudinal Section of the Strobilus.*—A median longitudinal section of the cone or part of the cone may be cut in pith or carrot. Though sections to show all the details require to be cut with a microtome, much can be made out with a hand section, which need not be very thin.

The central axis is broad and composed of relatively thin-walled cells (Fig. 32). The **sporophylls** are spirally arranged, each sporophyll being attached by a broad base which grows out almost horizontally and terminates in an upright leafy segment which becomes only one cell thick at the apex.

The **sporangia** are attached to the upper surface of the sporophylls near the base. The stalk of the sporangium is thick and is composed of a small-celled dense tissue with a vascular strand running through it. The sporangium is nearly spherical, its base being slightly tilted downwards away from the axis. There is no ligule. The wall of the sporangium is two cells thick, while the spores are small and possess slightly warted coats.

In comparison with *Selaginella*, it will be noted that all the spores are alike (**homosporous**) and that there is no differentiation into megaspores and microspores.

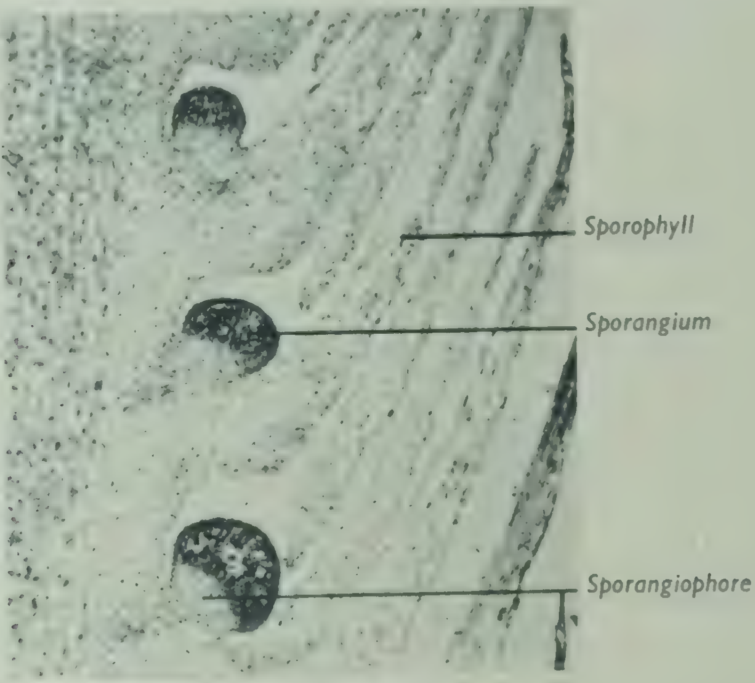

FIG. 32.—*Lycopodium clavatum.* Longitudinal section of several sporophylls.

2. **Selaginella.** *External Morphology.*—Although *Selaginella kraussiana* is a native of South Africa, it occurs so commonly in greenhouses and reproduces itself so readily, that fresh material should be easily obtainable.

The plant consists of a number of almost prostrate stems which branch dichotomously. One branch of the dichotomy always develops more strongly than the other, so that the appearance is almost that of a monopodial axis with side branches. At each bifurcation of the stem a colourless **rhizophore** is given off, which, when it reaches the soil, produces numerous endogenous roots which constitute the root system. The stem is clothed with oval, pointed leaves arranged decussately in four lines. In each pair of leaves one is small and one larger, and the large and small leaves alternate in successive pairs. The result is to produce two rows of large leaves, which spread out laterally on the stem, and two rows of small leaves, each opposite to a larger leaf, which lie close together on the upper side of the stem. Each leaf contains a single vascular bundle forming a midrib.

In older plants vertically growing shoots may develop at the ends of the horizontal stem. These differ from the others in that all the leaves are small and of equal size, and with a hand lens it is possible to see **sporangia** lying in the axils between the shoot and the leaves. These upright shoots, therefore, may be regarded as **strobili.**

There is not, as a rule, any primary root in mature specimens, for at an early stage this disorganises, and its place, as an absorptive organ, is taken by the adventitious roots from the rhizophores.

> *Transverse Section of the Stem.*—Cut a transverse section of the stem in pith or Carrot, stain in Aniline chloride and mount in Glycerine. It will be found advisable to cut fairly thickly or the central tissue may fall out.

On the outside there is an **epidermis** composed of small rectangular cells which are somewhat thick-walled. This is followed by a **cortex** composed of parenchymatous cells, forming a solid tissue three or four cells wide. In the centre are two **steles** which are separated from the cortex by a wide space, bridged only by a number of delicate, filamentous cells, widely separated. If a section is left to stain for some hours in very dilute Safranin it may be seen that each of these cells has a **Casparian band** about its middle. They are therefore interpreted as representing a modified **endodermis.** These filaments are very delicate and in a thin section may be easily broken, with the result that the central steles either fall out entirely or get so twisted that it is difficult to make out their structure.

The two steles agree in anatomical structure. Each is surrounded by a regular row of polygonal cells forming a **pericycle,** inside which is a more or less regular layer of small cells, the **phloem,** while in the centre is the **xylem** with one **protoxylem** group facing towards the outside of the stele. The structure is therefore **exarch.**

If a piece of stem is cleared for some time in Eau de Javelle it is possible to see that these steles give off tiny **leaf traces,** and that at each branch of the stem a part of each stele contributes to the formation of the two steles of the branch. Immediately below the branch origin, more or less in the angle between the main stem and the branch, the two steles of the stem unite, forming an arch which gives off from its upper side four arms, two of which pass into the branch and two continue up the main stem.

> *Transverse Section of the Rhizophore.*—Cut a transverse section of the rhizophore, using pith or Carrot ; stain in Aniline chloride and mount in Glycerine.

The **cortex** is bounded on the outside by a small-celled **epidermis,** which is heavily thickened, followed inwards by a series of thickened parenchymatous cells. On the inside are two thin-walled layers of non-thickened cells composing the **endodermis** and **pericycle.** In the centre there is a single group of **xylem** with a central **protoxylem** strand (**endarch**), and surrounding the xylem is a narrow band of **phloem.**

The whole structure of the rhizophore must therefore be regarded as extremely simple, and differs in no marked way from the root.

It should be noted that on reaching the ground the branches of the rhizophore develop **root-caps,** as may be seen by comparing a squashed preparation of the tip of an aerial rhizophore with one which has already rooted. Slides mounted whole after staining in Cotton Blue will show this point clearly. The underground branches of the rhizophore are therefore true roots.

Longitudinal Section of Strobilus.—Cut a median longitudinal section through the strobilus and mount in Glycerine. If a prepared slide cut by a microtome is available more detail will probably be made out.

The strobilus consists of a central axis which gives off **sporophylls** in alternating pairs (Fig. 33). The sporophyll is leaf-like, composed of several layers at the basal end, and tapering to a single layer at the apex. A branch of the main stele passes into it and forms a midrib. On the upper side of the sporophyll, close to the main axis, is a group of rectangular cells forming a small **sporangiophore** which supports the **sporangium.** On the side of the sporangium away from the axis is a little tongue of cells arising from the sporophyll, which is known as the **ligule.** Its function is unknown, but it may be merely vestigial, as it is well developed in the fossil forerunners of *Selaginella.*

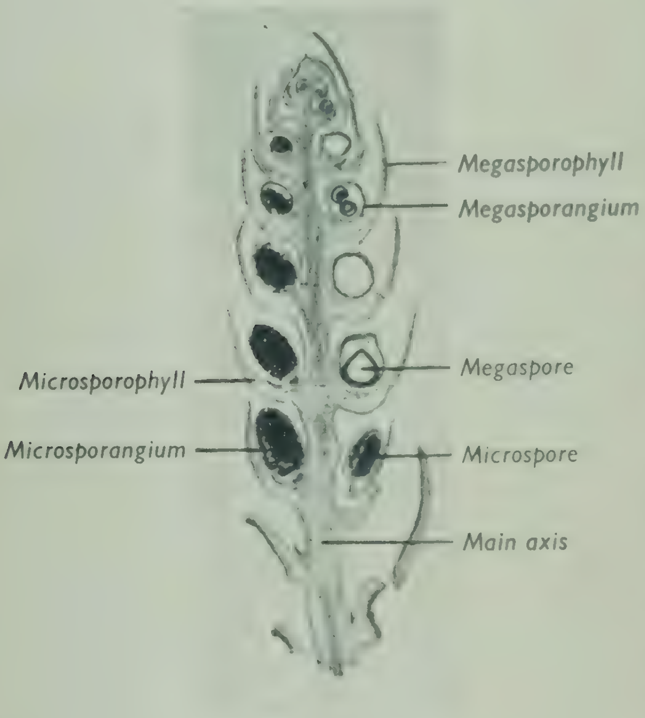

FIG. 33.—*Selaginella kraussiana.* Longitudinal section of the strobilus.

The sporangia are of two kinds, **microsporangia** and **megasporangia.** This condition is called **heterospory.** Each is provided with a wall composed of an outer layer of heavily thickened cells, whose radial and inner tangential walls are cutinized. Internal to this are one or two layers of the persistent **tapetum,** which may be made out in good sections. In the microsporangium the point of dehiscence may be seen as a break in the wall at the top of the sporangium, while in the megasporangium, which is larger and rounder than the microsporangium, a line of dehiscence runs right across the sporangium.

The **microspores** are numerous small rounded bodies, often grouped in fours, with thick, spiny coats which are heavily cutinized. The **megaspores,** of which there are only four in each sporangium, are oval bodies, each provided with a beak-like process. This beak indicates the point at which dehiscence will subsequently take place. It is the meeting-point of three slits in the spore coat. The coat splits open along these lines and the three flaps thus formed turn outwards to expose the contents. The microspores also open in a similar way. The wall of the megaspore is very thick, and is actually composed of several layers, the outermost one of which is cuticularized and has a rough warty surface.

It is improbable that any internal structure can be made out in the spores themselves, for their thick coats resist the penetration of many fixatives, and unless specially fixed, the contents soon disorganise.

If a section is taken through a young strobilus it is likely that instead of mature spores a certain amount of archesporial tissue will be found in the sporangia. In this case it will be possible to compare the small-celled tissue of the microsporangium with the single large megaspore mother-cell and by careful staining to see stages in the meiotic nuclear division.

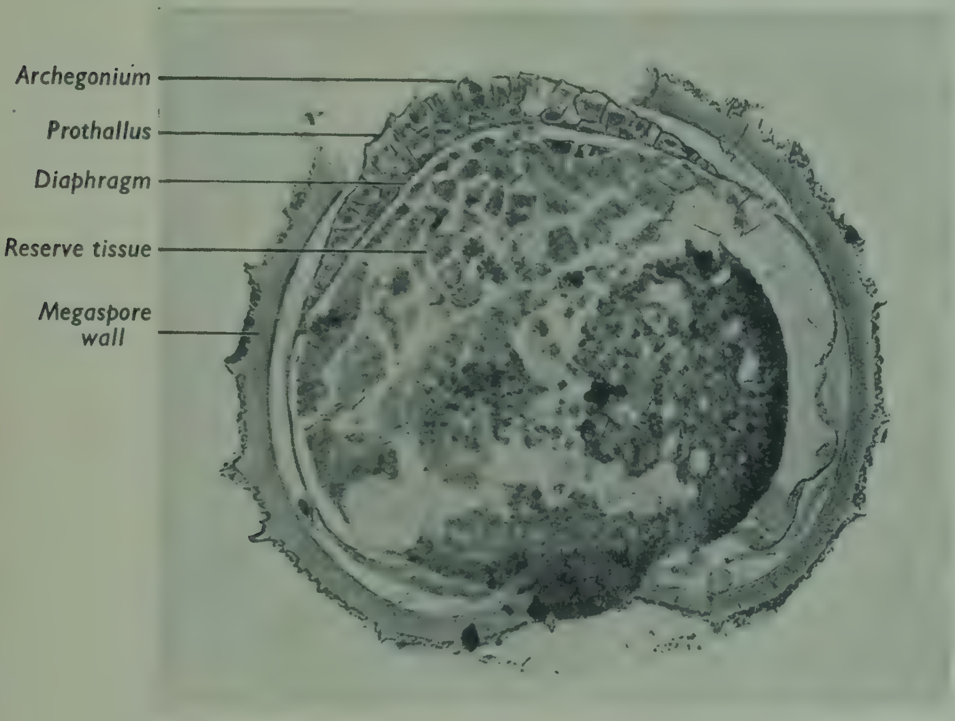

FIG. 34.—*Selaginella kraussiana.* Section through megaspore to show female prothallus.

Prothalli.—The student is scarcely likely to be able to prepare slides of these structures for himself, and the possibility of seeing the gametophytes must depend on what prepared slides he can obtain.

It must be remembered that in *Selaginella*, unlike previous types, the prothalli are retained within the wall of the spore, and that only sections of mature spores will show the structure. In the megaspore the tissue of the female gametophyte forms across the space exposed by the splitting of the megaspore wall (Fig. 34). The cell formation proceeds from the surface inwards, and on the surface the **archegonia** are developed. These are very simple bodies with a very poorly developed neck and venter, both embedded in the tissue of the gametophyte. Several may be formed on one prothallus, but only one develops an embryo.

The male prothallus is more difficult to see, even in a section of the relatively small microspore, but in a lucky section the division of the spore contents into central cells and peripheral cells may be observed. In the central cells a small number of **antherozoids** with two flagella are formed. The whole male prothallus is the equivalent of a single **antheridium.**

3. ***Isoetes.*** *External Morphology.*—Plants of *Isoetes lacustris* are not uncommon in pools and lakes on non-calcareous soils, especially in mountain regions. They grow submerged in shallow water and often form a continous sward.

The plants are small, usually 3 to 4 in., and seldom more than 6 in. high. The mature plant consists of a tuft of narrow awl-like leaves arising from a squat, black, tuberous stem about $\frac{1}{2}$ in. in breadth and rather less in height. From the lower side of the stem arise a number of thin dark-coloured roots which branch sparingly by dichotomy.

The upper part of the stem is entirely concealed by the broad winged bases of the leaves, which arise from the apex in two ranks but which later undergo some lateral displacement, so that they appear externally to form a close tuft in which divergences between $\frac{1}{3}$ and $\frac{8}{21}$ may be found between individual leaves.

Each leaf is potentially a sporophyll, and most of them bear sporangia which may be seen enclosed in the hollow leaf-base, which has an opening on the inner, concave side, partially covered by a flap of tissue called the **velum.** Above the velum may be seen the minute heart-shaped **ligule,** lying like a scale against the inner surface of the leaf. The outer leaves usually contain **megasporangia** and the inner leaves **microsporangia,** while the central immature leaves may be sterile, though they will persist until the following year and then develop sporangia. The plant is never devoid of leaves, though in the winter only sterile leaves may be present.

The exterior of the stem is blackish in colour, soft and irregular. It is somewhat flattened in the vertical direction and shows two broad shallow grooves opposite to each other, on the broader side of the stem, thus dividing the stem into two more or less rectangular lobes. These grooves are connected by a narrow groove across the base of the stem, on each side of which the **roots** are produced successively, forming two groups, with the youngest next to the groove. Careful observation will show that the roots are arranged in transverse rows parallel to the linear meristem which lies at the bottom of the basal groove.

Transverse Section of the Stem.—Cut a transverse section of the mature stem at about the middle level; stain in Aniline chloride.

The lobed outline of the stem is clearly seen in sections, the constriction produced by the two grooves giving the whole section the outline of an hour-glass. At the middle of the constriction lies the small **stele.**

The outer end of each lobe consists of a zone of empty parenchymatous cells, the outer layers of which are suberized and dark-coloured. This is the cortex of the previous year which is in process of destruction. The inner and larger part of each lobe consists of the current year's cortex, the cells of which are filled with starch grains. Through the cortex run the narrow **leaf-traces** radiating from the stele.

The stele itself consists of a central **hadrome** in which living parenchymatous cells are mixed with other parenchymatous-shaped cells which are devoid of contents and bear an irregular spiral band of lignin on their walls. These latter cells are apparently water-storage tracheids or **hydroids.** There is no regularly constituted xylem and no protoxylem. From the outer edge of the hadrome arise the leaf-traces, set so close together that eight or nine may appear in a single section. Between these traces lie masses of thin-walled, closely packed rectangular cells called the **prismatic tissue,** which is assumed to correspond to phloem, the individual cells being termed **leptoids.** The outer margin of this tissue is meristematic. It produces outwards a new cortex each year, displacing the old cortex, which soon erodes away. Inwards it adds to the leptoids. The whole stele is a primitive type of **protostele.** There is no endodermis.

> *Longitudinal Section of the Stem.*—There are two possible planes for longitudinal sections, namely, in the plane of the grooves and at right angles to this. The former is preferable. Remove all mature leaves and roots and cut a median section to pass through the growing point. Sections should be complete from top to bottom of the stem. Stain in Aniline chloride.

The stele appears slightly barrel-shaped in longitudinal section. It ends upwards in a truncated group of closely packed tracheids between which the parenchyma cells have not yet expanded to full size. The apex of the stele is separated from the growing point of the stem by a parenchymatous tissue, several cells thick, in which may be distinguished a meristematic zone forming a mantle over the top of the stele. Near the top there is no prismatic tissue around the hadrome. It starts about one-third of the way down and increases in width basiscopically by cambial activity. Numerous leaf traces are given off, over the whole length of the stele, each consisting of a central group of rather elongated spiral tracheids, surrounded by a zone of thin-walled leptoids. The leaf-traces leave the stele in the horizontal direction and later curve upwards to the leaf base. Those of former years cease inwards at the limit of the current year's cortex.

At the base the stele is expanded into a crescentic mass of tracheids, the two horns of which curve somewhat upwards. This is the **rhizophore,** and from it arise the roots, developed in sequence and directed outwards and downwards. They pass through the parenchyma of the lower or rhizophore portion of the axis and emerge from the base of the lobes on each side of the basal groove. A cambium mantle lies in contact with the lower side of the rhizophore, by means of which it grows secondarily.

The apex of the stem possesses no apical cell. It is somewhat concave, and the leaf primordia arise from it in two opposite ranks. In the youngest primordia it will be noticed that the ligule develops much faster than the leaf itself, and at one early stage actually overtops the leaf apex.

> *Transverse Section of the Leaf.*—Cut a transverse section about the middle of a mature leaf; mount in Glycerine.

Like most other water plants, *Isoetes* possesses large intercellular spaces in the leaves. The section is almost circular, though slightly flattened on the upper (adaxial) face. No specialized epidermis is present. There is a narrow peripheral belt of **cortex** surrounding four large lacunae which are separated by four diaphragms, each, like the cortex, three or four cells in thickness. These diaphragms meet at the centre, where a small mass of parenchyma surrounds the single vascular bundle. Each lacuna is also subdivided horizontally by numerous transverse diaphragms. No stomata are present though they occur on allied land-living species.

The **vascular bundle** itself is much reduced. Near the base of the leaf there are a few lignified tracheids in the centre, but below the middle level of the leaf these disappear and are replaced by a central cavity, occasionally with one or two subsidiary cavities beside it. These cavities are surrounded by a layer of cells which may show Casparian thickenings on their radial walls, though the layer can scarcely be regarded as the morphological equivalent of an endodermis. Outside this layer of cells is a zone of leptoids with somewhat thickened walls, forming a semicircle on the abaxial side of the bundle. There is no general endodermis around the bundle as a whole.

> *Transverse Section of the Root.*—The root is hollow and very delicate. Sections are most readily obtained if cut near the base of a young root of the current year.

Externally there is a piliferous layer with rather scanty root-hairs. The cells of this layer become suberized and yellow in colour, and form the permanent covering of the root. The cortex is a zone of parenchyma, three or four cells deep, surrounding a large central cavity. The single vascular bundle is attached to the inner surface of the cortex at one side of the central cavity. In the young root the bundle is central, but the formation of the large schizogenous space displaces the bundle to the side. The whole arrangement strikingly recalls the Carboniferous fossil root *Stigmaria*, which was attached to the lycopodialian tree, *Sigillaria*.

The vascular bundle consists of one or two central tracheids surrounded by a narrow band of leptoids and an endodermis with well-marked Casparian thickenings. The bundle is surrounded by the collapsed remains of a lacunar tissue which enclosed it in the young state and is retained only on the side of the bundle which adjoins the cortex.

> *Reproduction: The Megasporangium.*—The outer leaves usually bear megasporangia, which can be recognized by examining the base of the

leaf on the inner side. Megaspores, if present, can be seen with a hand lens, appearing as whitish spheres through the sporangium wall. Cut a longitudinal section through the base of the megasporophyll, in the direction radial to the axis. It is best to choose a young megasporophyll in which the spores are still soft.

The megasporangium is surrounded by the tissues of the sporophyll, lying in a cavity, the **fovea,** to the back of which it is broadly attached. An outgrowth of the leaf tissue called the **velum** forms a partial covering over the outer side of the sporangium. Above the sporangium lies the **ligular pit** from which arises the **ligule** with its large-celled base or **glossopodium.** Above this extends the leaf proper with its large lacunae crossed by transverse diaphragms. If the section is truly median the vascular bundle of the leaf trace will be seen, passing behind the sporangium into the median shaft of tissue in the leaf base. Spiral tracheids may be distinguished in it to a level somewhat above the top of the sporangium. Two irregular strands of tracheids extend laterally from the leaf trace, one into the top of the velum and the other above the ligular pit. These may be regarded as **transfusion tissue.**

The wall of the sporangium is two to three cells thick, the inner being a transitory **tapetum.** The lumen of the sporangium is divided irregularly by a small number of **trabeculae** of sterile tissue. The sporangium as a whole contains about forty-eight megaspores which measure 0·6 to 0·8 mm. in diameter and show a number of wall layers. Firstly, each megaspore is surrounded by a **special mother-cell wall,** which develops after meiosis, dividing the mother cell into four cells, each containing a megaspore. This wall may later become very swollen, and finally it disintegrates. Within this wall lies the thick yellow **exospore** and within this again is a thinner, paler layer, the **mesospore,** which lies close to the protoplasm and is, in the earlier stages, shrunk away from the exospore. Later it expands into contact with the exospore, and a thinner pectic layer, the **endospore,** makes its appearance around the cytoplasm. The latter is abundant, but is extremely spongy in structure and shrinks easily to one side of the spore, taking the megaspore membrane with it. It contains an abundance of droplets of fatty oil.

The megaspore, seen as a whole object in the mature state, shows a rough surface with a tri-radiate ridge which extends to the equator of the spore, where its arms join a transverse ridge which runs round the equator.

Reproduction : *The Microsporangium.*—Select a fully grown leaf from near the middle of the tuft and cut a radial longitudinal section through the base, including the sporangium ; mount in Glycerine.

The details of the leaf structure are identical with those seen in the section of the megasporophyll described above. The sporangium itself, however, contains an immense number of minute brownish **microspores** somewhat spindle-shaped, with three pronounced ridges running from end to end. The number of microspores in a single microsporangium is larger than in any other plant and may amount, in some cases, to as many as a million.

Section 10. Gymnospermae

1. **Pinus.** *Morphology of Branch.*—The branches of *Pinus sylvestris*, the Scots Pine, grow in whorls from lateral buds which are formed in the axils of scale leaves at the end of each year's growth. As the branches usually develop about equally, the tree in the young state presents a very regular pyramidal shape.

The examination of a single branch will show that it bears numerous spirally arranged **scale leaves,** in the axils of which secondary branches of very limited growth are produced. These are the short shoots or **foliage spurs.** At the top of the branch of the current year the foliage spurs will be seen most clearly. If these are examined closely they will be seen to bear the young foliage leaves in pairs.

Each foliage spur is covered at its base by scale leaves, and ends in a pair of **foliage leaves** or " needles." Other species of Pine may be found to have the foliage leaves produced either singly or in larger numbers than two, a point which is made use of in classification, but they are in all cases borne on foliage spurs.

The Pine is an evergreen, that is to say, not all the leaves are shed at the same time, as in the Larch, but persist for several years, being shed in small numbers throughout the year, so that, although leaves are being more or less continuously shed, new leaves are also being produced, and the tree appears always covered with leaves.

The **male cones** are produced in the axils of scale leaves at the base of the developing shoot of the current year, thus replacing foliage spurs. These male cones usually appear about May. They are soft, yellowish and about one-third of an inch long and are found in dense clusters.

The **female cones** are also developed laterally in the axils of scale leaves, but are produced in place of branches towards the apex of the developing shoot, and on different branches from the male cones. The young female cones appear singly or in small groups. They are at first pinkish-green in hue and are harder than the males, even in the youngest stage.

Transverse Section of the Young Stem.—Cut a transverse section through a first year stem, stain in Aniline chloride and mount in Glycerine.[1]

In a section through a stem of this age may be seen the typical primary stem structure before secondary thickening has occurred. The **epidermis** lies on the outside of the section and is somewhat cuticularized on its outer tangential walls. It is composed of small cells which are rectangular in shape. There is a wide cortex composed of loosely packed, round parenchyma cells with many intercellular spaces. One or two rings of **resin canals** occur, each canal being lined on the inside by a layer of small secretory cells whose

[1] If available, *P. parviflora* may be used for this section, as its young shoots are devoid of foliar spurs for considerable lengths, thus showing an uncomplicated vascular structure.

inner walls bulge into the cavity and from which the resin is secreted. This layer is known as the **epithelium.** Surrounding these canals outside the epithelium is a layer or two of small, rectangular, cortical cells. Considerable tannin deposits occur in the cortical cells (p. 48).

The vascular system consists of a series of **vascular bundles** separated by **medullary rays** which may be more than one cell wide. Each bundle consists of a mass of **xylem,** composed of an internal group of protoxylem elements followed by regular rows of metaxylem. The primary **phloem** consists of small sieve tubes and is separated from the xylem by a small quantity of procambial parenchyma from which the **cambium** is differentiated.

There is a solid, central, parenchymatous **pith** whose cells contain starch grains.

> *Transverse Section of the Older Stem.*—Cut a transverse section of the stem after the commencement of secondary thickening, stain in Aniline chloride and mount in Glycerine.

Due to the development of the **cork cambium** in the outer zone of the cortex, **cork** is laid down in successive layers around the outside of the stem, and at the same time a small quantity of **secondary cortex** is formed consisting of regular radial rows of rather loose parenchyma tissue, laid down inside the cork cambium ring. There is a regular ring of resin canals in the primary cortex. Each is elliptical in outline and is lined by an epithelium, as described in the last section.

The tissues of the stele consist of a narrow ring of **secondary phloem** and a large amount of **secondary xylem,** separated from one another by a **cambium.** Primary and secondary **medullary rays** pass through this tissue, the primary rays passing from the pith to the cortex. The secondary rays do not all go the whole distance, some being newly formed in each annual ring. The ring of primary bundles can be made out by the groups of protoxylem and metaxylem lying internal to the secondary wood and embedded in the edge of the pith. Compared with many Angiosperms the pith is large. **Resin canals** in the secondary wood occur scattered in a series of rather irregular rings around the stem.

Analysis of the elements will show that practically the whole of the secondary xylem is made up of **tracheids** with **bordered pits** on the radial, and to some extent on the tangential walls. These bordered pits are characteristic of *Pinus.* They are somewhat like blisters on the cell walls, and are developed by the outgrowth of the lignified wall of the cell on both sides of the middle lamella to form a dome, hollow in the centre. Since similar domes are formed on both sides of the lamella, a hollow circular space is formed, traversed only by the middle lamella. This cavity has two holes opposite one another, while in the centre, on the middle lamella, there is developed a small plug, the **torus.** If pressure becomes too great on one side the middle lamella is pushed over and the torus closes the opening in the pit of the far side, thus preventing the passage of water. We shall refer again to bordered pits in our consideration of the radial and tangential longitudinal sections.

It should be noted that neither wood parenchyma nor vessels occur in the secondary wood of *Pinus*.

In a more mature stem, several years old, the same structures will be seen, but in addition it is possible to make out the annual rings of growth. These are clearly marked by the unequal size of the small tracheids formed in autumn and the larger ones produced the following spring. This sudden change in diameter is clearly recognizable and the line of junction of the two types produces a clearly marked ring.

> *Radial Longitudinal Section of the Older Stem.*—Cut a longitudinal section through the centre of the stem, thus making the section lie along the diameter of the wood as viewed in transverse section.

In a longitudinal section of this kind the **cork cells** of the exterior are seen as thick-walled prosenchyma, being bounded on the inside by the narrow elongated cells of the **cork cambium.** The **cortex** is made up of parenchymatous cells with intercellular spaces, traversed here and there by longitudinally running **resin canals,** with lining **epithelial cells** which stain up brightly. These ducts run roughly parallel through the cortex. The secondary tissue is made up on the outside of sieve tubes with oblique end walls, followed inwards by the much thinner **cambium** elements. The **xylem** consists of tracheids, each with a single row of bordered pits, which in such a section appear as two concentric rings, the outer ring representing the outer limit of the pit and the inner ring the opening to the cavity containing the torus. Towards the centre of the section may be seen the **primary wood**, which is composed of tracheids with spiral or with reticulate thickening. In the centre of the section is the large-celled, parenchymatous **pith.**

The **medullary rays** are the most conspicuous feature in the section. In a radial section the medullary ray will be cut longitudinally. It is therefore possible in a radial section to study this important aspect of the ray. It will be noted that the structure of the ray differs in the region of the phloem and in the xylem. In the region of the phloem the ray is bounded above and below by cells elongated in the direction of the phloem elements. These are termed the **albuminous cells.** They are thin-walled and contain food reserve material and large nuclei. The central part of the ray is composed of starch-containing cells of more rectangular shape. In the region of the xylem there is a similar central core of oblong lignified cells containing starch which passes along the centre of the ray, and on each side of this are short horizontal **ray tracheids** whose walls are supplied with bordered pits. In the section some of these pits will be seen to be cut longitudinally.

Note that the pits between the central ray cells and the tracheids of the xylem are not bordered but simple. Their number per cell varies characteristically in different species of *Pinus*.

Tangential Longitudinal Section of the Older Stem.—Cut a longitudinal section of the stem passing through the outer part of the woody cylinder, stain in Aniline chloride and mount in Glycerine.

The chief feature of interest in the tangential section relates to the appearance of the medullary ray, for most of the other tissues will appear essentially similar both in radial and tangential section. In the tangential section the medullary ray will be cut transversely, and therefore, by comparison with the transverse and radial sections, it is possible to build up a three-dimensional conception of the appearance of the ray.

In tangential section the ray appears as a short row of more or less rounded cells, three or four cells high, of which the central cells may be slightly broader than the others. If the section has been taken through the phloem, the peripheral cells of the ray, the albuminous cells, will appear rather elongated. In the xylem portion the marginal tracheids of the ray may be easily distinguished by their lack of cell-contents as compared with the central cells which contain starch grains. Here and there will be found larger rays of up to twelve cells high. These **fusiform rays** differ from the smaller ones in having a general oval shape, brought about by the fact that the central part of the ray is two or three cells wide instead of being composed of a single row of cells. There may also be sometimes a horizontal resin canal included in these larger rays.

The xylem tracheids will be seen to have only a very few, irregularly arranged, bordered pits lying on their tangential walls, and therefore seen in surface view, as well as the numerous pits on the radial walls, which will be cut longitudinally. In this way the complete structure of a pit can be seen in the same section.

Transverse Section of the Root.—Cut a transverse section through a young root, stain in Aniline chloride and mount in Glycerine.

Even at a young stage secondary thickening occurs, including the formation of a **cork cambium** which destroys the primary tissues of the cortex and leaves the roots surrounded on the outside by a layer of **cork,** followed by a number of rows of rather rounded cells forming the **pericycle.** It follows, therefore, that the most noticeable character of the root is the tissues of the stele. On the outside is a narrow zone of **secondary phloem,** composed of very narrow small sieve tubes, which are separated from the **secondary xylem** by the **cambium.** The secondary xylem is composed of **tracheids** arranged in rather regular rows and interspersed with uniseriate **medullary rays.** In the immediate proximity of each protoxylem group is a large **resin canal** which is very characteristic of pine root-wood. The **primary xylem** consists of a single central plate of metaxylem with the two protoxylem groups situated at the two ends. There is consequently no pith. Between the secondary phloem and the pericycle may be seen, especially in young roots, a certain amount of crushed tissue, which represents the remains of the primary phloem. While the **diarch** structure described above is typical of the youngest roots, some may be found which are **triarch.** Their anatomy is, however, essentially similar.

A careful examination of the walls of the xylem tracheids will show that, while in the first few rows pitting may occur on all walls, in the later formed xylem pits are developed only on the radial walls.

In an older root section it will be found that a complete ring of xylem is developed by the spread of the cambium outside the two primary resin canals, and that additional resin canals appear in the secondary xylem, often in conjunction with small islands of parenchyma and medullary ray tissue. The primary xylem, however, still remains separated from the secondary xylem by bands of conjunctive parenchyma.

> *Transverse Section of the Leaf.*—Cut a transverse section through the leaf, stain in Aniline chloride and mount in Glycerine.

The section shows a characteristic shape in transverse section, for the adaxial surface is flat, while the abaxial surface is semi-circular. It should, however, be noted that the shape of the leaves in different species may not be the same.

The leaf shows typical xeromorphic structure, with a very much thickened **epidermis** and **cuticle** and deeply sunken **stomata,** lying at the base of narrow grooves. Below the epidermis is a thickened **hypodermis** comprising about three layers of thickened cells broken at intervals by the guard cells of the stomata and the stomatal cavities.

The next tissue is the **mesophyll,** which is composed of closely packed cells, the walls of which show remarkable " infolding " or invagination, by means of which the total internal surface of the cell available for the accommodation of chloroplasts is increased, just as a librarian increases his book space by placing bookcases at right angles to the wall. These invaginations are probably not true ingrowths of the wall, but represent lines where the growth of the cell has been prevented by local wall thickening, so that the wall has bulged out between them, leaving them behind. The layer next to the hypodermis has usually much deeper invaginations than the others, so that the cells appear to be divided into arms.

There follows a well-marked **endodermis** with a clearly seen **Casparian band** running round each cell. Within this lies a mass of **transfusion tissue** with two vascular bundles in the centre. The transfusion tissue is composed of two distinct parts. One is aligned alongside the xylem and consists of empty parenchyma cells, with bordered pits like those of tracheids, while the other part is associated with the phloem and is formed of small cells with dense contents rich in protein.

The vascular bundles lie obliquely to each other, and each is composed of a mass of **xylem** with narrow rays of parenchymatous cells running through it, and a group of very small regular **phloem** cells.

> *Longitudinal Section of the Leaf.*—Cut a longitudinal section, or a series of sections, through the leaf; stain in Aniline chloride and mount in Glycerine.

The use of longitudinal sections will assist in the understanding of the leaf of *Pinus*. On the outside are the regular rows of the epidermis and hypodermis, broken at intervals by the stomata with their guard cells sunken into little cavities, down to the base of the hypodermal layer. The mesophyll with its curious invaginated cells is clearly seen, and it will be noted that in this view it appears as a series of cell-plates, transverse to the leaf, with large transverse intercellular spaces between the plates. These spaces are connected with the stomata.

The **endodermis** is composed of much elongated, large cells which stand out now quite clearly from the **transfusion tissue.** On the side of each endodermis cell may be seen the **Casparian band** with a median row of simple pits. The large spherical **albuminous cells** of the transfusion tissue may be noted. If the section passes through the vascular bundle it will be seen that the first formed **xylem** has tracheids with spiral thickening, while the **metaxylem** is formed of pitted tracheids. The sieve tubes with their sieve plates may also be made out on the **phloem.**

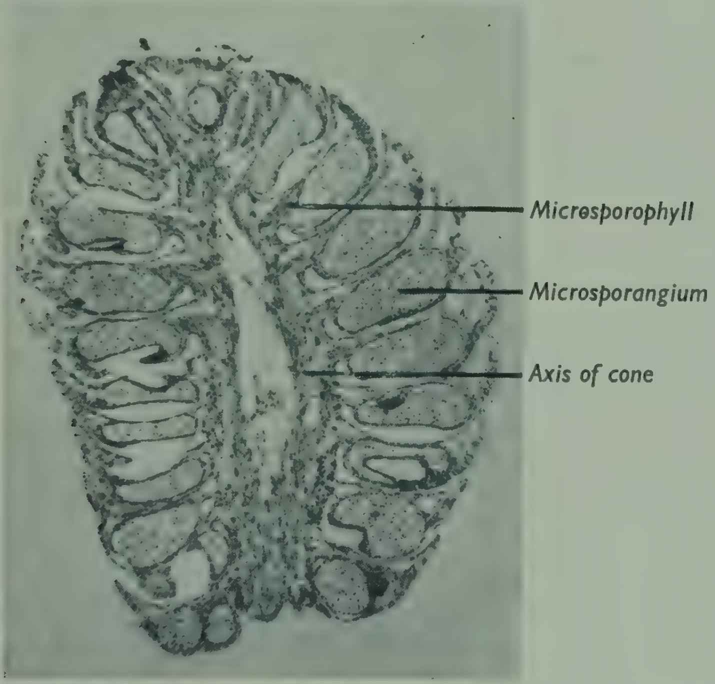

FIG. 35.—*Pinus sylvestris.* Longitudinal section of male cone.

> *Male Cone.*—The male cone is borne in place of a short shoot in the axil of a scale leaf. It is an elongated oval structure, $\frac{1}{4}$ to $\frac{1}{2}$ in. in length, consisting of a central axis on which are borne the sporophylls, each of which bears two sporangia on its lower surface. Cut a longitudinal section through a young cone and examine under the microscope.

The **central axis** is rather thick and, if the section is median, will show a typical stem structure with a vascular cylinder and pith in the centre (Fig. 35).

The sporophylls are arranged on the axis in a close spiral. Each **sporophyll** is a flat structure, the distal end of which is prolonged both upwards and downwards, overlapping the sporophylls above and below. The **sporangia** are long and are attached to the sporophylls along their whole length. Each sporangium is surrounded by a single wall of cells, though if young the **tapetum** layer which occurs inside may also be visible.

The appearance of the contents of the sporangia will depend on the age of the cone. In a very young cone this may be made up of the **archesporial tissue** from which the **pollen mother-cells** become differentiated. By division of the pollen mother-cells into four, during which reduction division occurs, the pollen grains are formed. If the cone is mature each sporangium will be filled with ripe **pollen grains** or **microspores,** each consisting of a large **vegetative** or **tube cell,** while a small **prothallial cell** may be distinguishable beside it, against the wall. On the sides of the grain two wing-like sacs occur. These are formed by the swelling and separation of the cuticularized exine from the cellulose wall inside. These wings assist in the dissemination of the pollen grains by the agency of the wind and in orientating them in the ovule, in relation to the nucellus.

> *Morphology of the Female Cone.*—The mature cone is a pointed oval structure, composed of a number of woody scales, each of which is attached at its proximal end to a central woody axis. Below each scale is a smaller scale, or bract, which subtends the larger ovuliferous scale.

The scales are wedge-shaped in surface view, widening out at the distal end into more or less rhombic plates. These plates fit closely together at their margins and increase in size as one passes from the base to the middle of the cone. In the first and second years the cone is green and the scales are closely pressed together, with the result that the cone as a whole assumes an elongated shape. In the third year it turns brown, but up to this time the scales remain in close contact, except for a short while during the period of pollination.

In the third year the scales separate, allowing the seeds to escape. At this stage it is easy to see that the proximal part consists of a flattened arm, bearing on its upper surface two **seeds,** attached at the proximal end of the scale. The distal end of each seed is continued in a thin membranous **wing** which lies close against the upper surface of the scale. These wings lie side by side, their adjacent edges being parallel and their outer edges curved, so that together they cover the upper surface of the scale.

At the apex of the cone may be one or two sterile scales. The seeds are shed from between the scales and flutter down to the ground, being blown about by the wind as they fall, a process which is aided by the wing. After the seeds have been shed the cone itself ultimately falls to the ground.

> *Longitudinal Section of First-year Female Cone.*—Cut a longitudinal section through a young female cone, stain in Aniline chloride and mount in Glycerine. As this and the sections of the female cone to be

described subsequently are difficult to cut, microtome sections may be substituted with advantage, though much can be made out from a good hand section taken from a cone of appropriate age.

There is a wide central axis supplied with a vascular system similar to that in the stem, which sends out branches to the scales. Each scale is a double structure. There is an upper, massive, woody scale bearing the ovules, as described above, and a lower small and leathery scale close beneath the larger one. The homologies of these structures have been the subject of considerable controversy, but for the purpose of this book the large structure on which the ovules are borne will still be called the **ovuliferous scale,** while the structure developed below will be referred to as the **bract scale.**[1]

Attached side by side to the proximal end of the ovuliferous scale are the **ovules,** one of which may appear in section. The amount of detail to be seen in the ovule will depend on the quality of the section. It should, however, be possible to recognize the **single integument** enclosing a narrow **micropyle** at the apex. Inside this at the upper end will be seen the **nucellus,** while lying in the parenchymatous tissue of the nucellus is the **embryo sac** containing the **female prothallus** with two large **archegonia** at its upper end.

A transverse section of the ovuliferous scale taken near its base will show the two ovules lying side by side on the upper surface of the scale, and it will be noticed that the surface of the scale is not flat but curved to fit the shape of the developing ovules. Such a section may be made with advantage to compare with the longitudinal section. It also shows an arc of collateral vascular bundles which are inversely orientated, that is, with the xylem directed towards the lower, abaxial, surface of the scale, *i.e.*, the reverse of the arrangement in leaves and bracts.

Longitudinal Section of the Ovule.—The preparation by hand of a slide to show the details of the ovule development requires more skill than can be reasonably expected of students, and microtome sections are almost essential.

In a longitudinal section through a young ovule may be seen the single **integument,** the outer layer of which consists of closely packed cells which are densely filled with protoplasm and in the inside more irregular tissue composed of more vacuolate cells. The integument is fused to the **nucellus** from the base to about half-way up, but above that it is free and somewhat separated from the nucellus. The innermost layer of the integument is somewhat thickened. The nucellus is composed of small cells with well-marked nuclei, and is filled with food material and protoplasm. At the apex it forms a triangular wedge of tissue which pushes up into the lower end of the micropyle.

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[1] See "Textbook of Theoretical Botany," vol. i, p. 677 *et seq.* McLean and Cook. Longmans, Green & Co. Ltd.

The **embryo sac** is an oval body lying in the lower part of the nucellus, its upper end reaching to approximately the point where the integument becomes fused with the nucellus. It contains mainly a loose parenchymatous tissue, the **prothallus,** which at maturity consists of uninucleate cells with scanty cytoplasm. At the upper end of the embryo sac lie the two **archegonia** (Fig. 36). If the section is truly longitudinal it may be possible to see the neck cells, which are formed at the upper end, but in all probability the only visible structure will be the large **oosphere** which terminates the archegonium. This oosphere is densely full of cytoplasm, and its single, large nucleus can be easily seen. The neck consists of one row of four cells, grouped in a square, with an intercellular space in the middle.

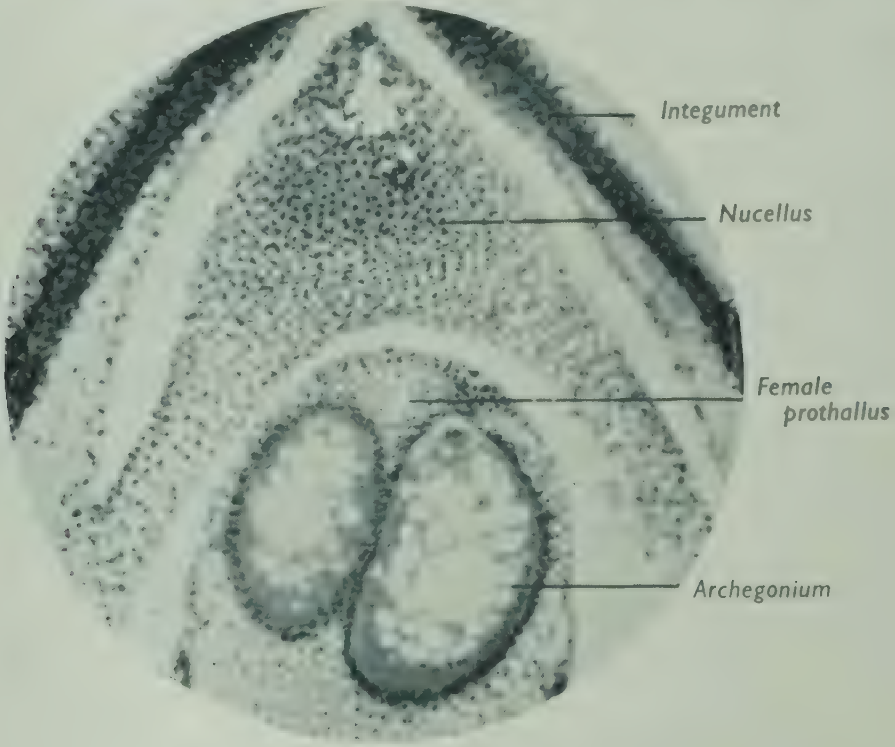

Fig. 36.—*Pinus sylvestris.* Vertical section of the ovule showing archegonia.

> *Longitudinal Section of Ovule with Embryo.*—Such a section can be obtained by hand from a two-year-old pine cone, but satisfactory detail can best be obtained by using a microtome section.

In such a section it is possible to see that, as a result of the fertilization of the oosphere of one of the archegonia, an embryo has been formed, and that a group of very elongated cells, the **suspensors,** has developed, pushing its way down among the cells of the endosperm, which by this time has become filled with food reserve material. The **embryo** proper develops at the lower end of the suspensors, surrounded on all sides by the prothallus.

It is improbable that any details can be made out regarding the structure of the developing embryo, but it is desirable to note that the embryo proper develops outside the boundary of the archegonium, and the elongation of the cells of the suspensor, which may be visible, tends to push the embryo farther and farther down into the tissues of the prothallus.

Longitudinal Section of a Mature Seed.—Crack and remove carefully the shell of a mature Pine seed and cut the contents in half lengthways.

The young **embryo** will be seen to consist of a **radicle** or root, directed towards the micropyle, while the **hypocotyl** makes up the greater part of the embryo. It is surmounted by the group of thick **cotyledons,** which, if a transverse section is cut at this point, will be found to number about eight. In the centre is the beginning of the young **plumule.**

The whole embryo is embedded in **prothallial tissue** and enclosed in a woody seed coat to which the membranous wing is attached.

At the time of germination the radicle grows out of the micropyle, the cotyledons absorb the remainder of the endosperm, the testa splits open and the tiny pine seedling begins an independent existence.

Young Seedling.—The young seedling Pine shows a number of interesting points.

There is a short permanent root which gives off lateral roots. Above the ground the most noticeable structure is the **hypocotyl,** which is surmounted by a crown of about eight **cotyledons.** These are green in colour and resemble the foliage leaves. In the centre of this lies the young stem, which later grows out as the main stem of unlimited growth, and forms as it grows ; first, **juvenile foliage** leaves which are not developed on foliar spurs but attached directly to the stem in spiral order. After a short while, however, the first **foliar spurs** develop in the axils of the juvenile leaves, and the typical Pine foliage is produced. As growth continues the juvenile leaves are replaced by scale leaves which are found on all branches of the mature plant.

2. **Taxus.** *External Morphology of the Shoot.*—The Common Yew (*Taxus baccata*) grows to a considerable size and is represented by a tree common in this country both as individual specimens and in homogeneous woods on calcareous soils. The wood is tough and withstands rotting, so that it is largely used for outdoor constructional work. The strength and pliability of the wood was previously made use of in the making of bows.

Examine a branch and note that the leaves appear to be arranged in two rows, arising directly from the branch without the formation of foliage spurs. The leaves are in fact spirally inserted, but a twist at the base of each makes them lie in two ranks one on each side of the stem. The leaves are narrowly oblong with a definite base and terminate in a sharp point. The cones are borne in the axils of the foliage leaves. In the case of the male cones their stalks turn down so that the cones hang along the lower side of the branch.

The female cones are borne at the top of a branch in the axil of one of the upper leaves.

A mutant variety of the common Yew, known as the Irish Yew, is characterized by having a slender upright habit with spreading leaves. A brief account of this is given in Part X, p. 441.

> *Transverse Section of the Stem.*—Cut a transverse section of the young stem, stain in Aniline chloride and mount in Glycerine.

The various tissues may be conveniently compared with the corresponding features in *Pinus*. On the outside is a narrow zone of **cork cells** which are usually narrow and produced in regular rows from a **cork cambium.** These cells are suberized, and if the material is very young, some remains of the primary cortex may be seen on the outside. A small amount of **secondary cortex** may be present or this may be entirely wanting, and the inner primary cortex may reach right up to the cork cambium. This **primary cortex** is composed of rather rounded parenchymatous cells, with a certain amount of air space between them.

The **secondary phloem** will be seen, composed of regular rows of thin-walled cells traversed by uniseriate **medullary rays.** The phloem is separated from the **secondary xylem** by a **cambium** which is sometimes clearly visible. The secondary xylem is made up of very regular, rather small tracheids, with spiral thickenings on them. This mode of thickening, which is reminiscent of protoxylem, affords great pliability and explains the selection of the Yews for bows, which must be pliable as well as strong.

Uniseriate **medullary rays** pass through the xylem and between the primary bundles which border the well-developed **pith.** By the difference in the size of the tracheids produced in spring and autumn, it is possible to recognize successive annual rings of growth.

Taxus wood is easily distinguished from that of *Pinus* because of the absence of resin canals in the former genus.

> *Transverse Section of the Root.*—Cut a transverse section through a young root, stain in Aniline chloride and mount in Glycerine.

The section may be compared with that of *Pinus* from which it differs only in a few points.

There is a well-developed external **cork layer** composed of cells derived from an active **cork cambium** and containing much tannin deposit. Inside is a certain amount of cortical tissue which resembles closely that found in the stem.

The amount of **secondary phloem** produced is relatively small, and is out of all proportion to the **secondary xylem,** which is extensive and composed of very regular tracheids. Uniseriate **medullary rays** run through the secondary tissue.

As in other Gymnosperm roots there are typically only two **protoxylem** groups with a **metaxylem plate** between them. In *Taxus*, unlike *Pinus*, the protoxylem persists and is not displaced by a resin canal. In fact, resin canals are absent from the entire root as they are from the stem.

Transverse Section of the Leaf.—Cut a transverse section of a mature leaf, stain in Aniline chloride and mount in Glycerine.

In section the leaf appears flattened and symmetrical about a single central **vascular bundle,** embedded in **sclerenchyma,** with a large **resin sac** lying immediately below the phloem. There is a small but regular group of xylem and a somewhat larger group of phloem elements below it. On either side of the bundle is a small amount of **transfusion tissue.**

The leaf is surrounded by a thick-walled **epidermis** followed inwards by a less regular thickened **hypodermis.** The **palisade tissue** consists of a single layer of cells, immediately below the hypodermis, which passes indefinitely into the spongy mesophyll towards the lower surface. The whole mesophyll tissue, however, is made up of rather rectangular cells, containing chloroplasts and interspersed with air spaces. At the margin of the leaf even the palisade tissue may be somewhat thickened. **Stomata** are restricted to two lines along the lower surface, which are marked by two white bands of wax on the under side of the leaf as seen by the naked eye.

Male Cone.—The Yew is dioecious, the male and female " flowers " being produced on different trees. The male cone is borne in the axil of a leaf of the previous year and appears in February and March. As a result of growth of the axis the male cones are turned to the underside of the branches.

The cone, which is only a few millimetres long and whitish in colour, consists of a central **axis** bearing about six to ten **sporangiophores,** below which are a number of **scale leaves** which, in the early stages, cover over and protect the young **microsporangia**.

Cut a longitudinal section of the cone at an early stage, when the following tissues can be made out.

The sporangiophores are arranged in an umbel around the upper end of the central axis, with no subtending bracts (Fig. 37). Each sporangiophore is umbrella-shaped and bears a number of sporangia arranged round the stalk, not more than two of which will be seen in any one section. These sporangia are adherent to the sporangiophore along their entire length and possess a single wall which is made up of thickened cells. It will depend on the age of the sporangium whether the content is **archesporial tissue** or mature **spores.** If the latter, they will be seen to consist of small, spherical, smooth-walled bodies, each containing a single nucleus. Around the inside of the wall will be seen the remains of the **tapetum,** which has become largely disorganized.

Female Flower.—Unlike the male cone, the female organ is reduced to a single ovule, borne at the apex of a short shoot, which bears a number of overlapping scale leaves. Cut a longitudinal section through the ovule and mount in Glycerine.

This bud-like shoot is formed in the axil of an upper leaf of the previous year. The apex of the bud undergoes no further development, but in the axil of one of the uppermost scale leaves there develops a very short lateral shoot which itself bears a few scale leaves and terminates in a single **ovule** which constitutes the female flower. As the ovule grows it pushes aside the vegetative apex of the bud and assumes a terminal position.

On the outside is a very heavily thickened **epidermis** lying immediately over a layer of very large rectangular cells which are filled with solid deeply staining material (Fig. 38). These cells later form a stony coat in the testa of the ripe seed. Inside is the thin-walled tissue of the **single integument,** which is bounded on the inside by a layer of very elongated narrow cells also containing stainable material. Between the lips of the integument is a narrow **micropyle.** The centre of the ovule itself is made up of a thin-walled **nucellus** with a single central **embryo sac.** The nucellus is attached to the integument only near the basal end. Two **vascular bundles** enter the base of the seed and pass upwards through the soft, inner tissue of the integument. There is no vascular supply to the nucellus.

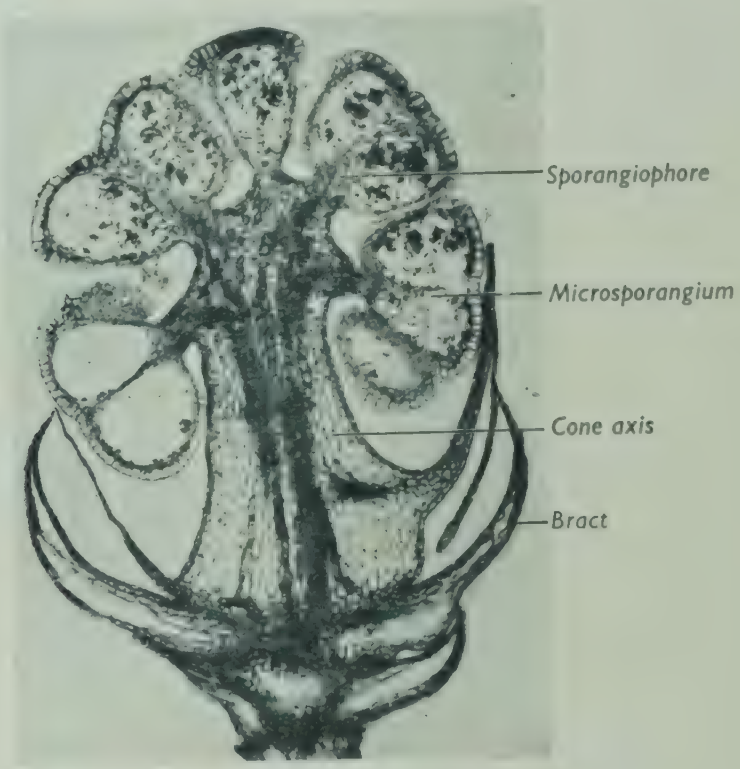

FIG. 37.—*Taxus baccata.* Longitudinal section of male cone.

At the base of the nucellus are numerous deeply staining cells containing tannin material, and in this region the cells are very small and densely packed together, suggesting an area of active meristematic division.

Outside and around the base of the integument there arises a tissue which grows rapidly and develops into the **cupule,** a soft structure which in the mature flower will surround the developing ovule. The mature cupule is bright red in colour and contains cells rich in sugars. It is edible and becomes attractive to birds, thus assisting the distribution of the seeds.

Below the zone from which the cupule develops there are a number of sterile leaves which will appear cut longitudinally in the section.

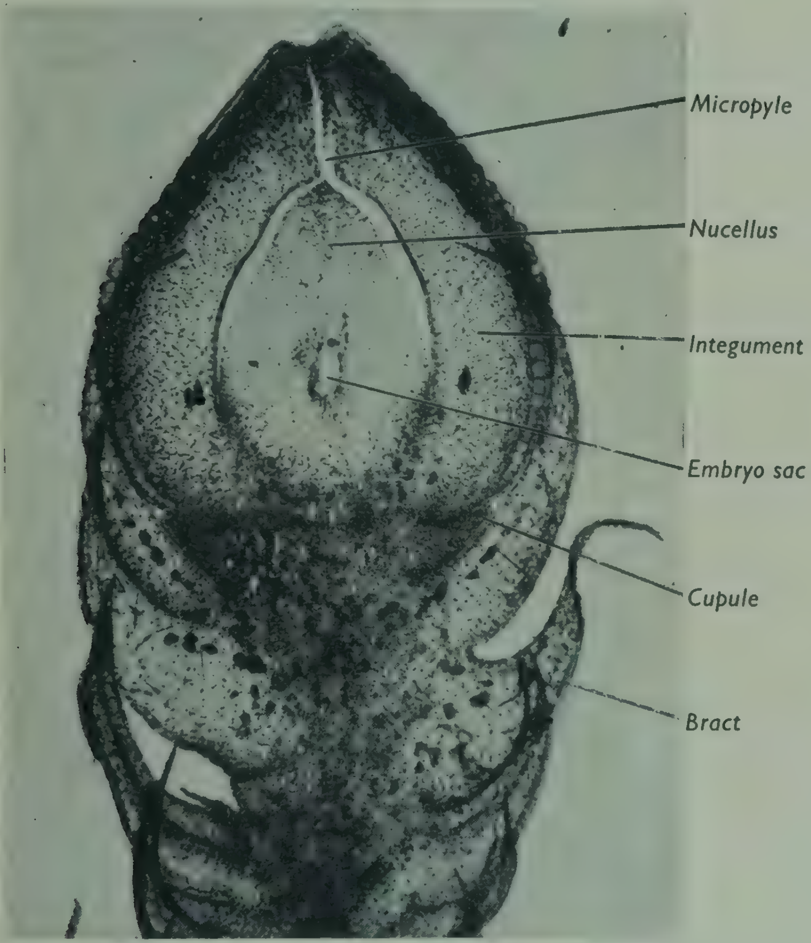

FIG. 38.—*Taxus baccata.* Longitudinal section through the ovule.

3. ***Cycas revoluta*** (Fig. 39). *Morphology of the stem.*—If a museum specimen of a part of the stem of a member of the Cycadales is available an attempt can be made to study its anatomy.

Typically the stem is unbranched and, in its natural habitat, varies in length up to 20 or 30 ft. in a few old specimens. The stem is crowned at the top by a cluster of large leaves which have caused the group to be popularly confused with the Palms.

The trunk is covered with an armour of hardened **leaf bases,** for unlike the leaves of Angiosperms, those of *Cycas*, when dying, first lose their leaflets, after which the petiole bends down and begins to decay and an abscission layer is developed which leaves a few centimetres of the petiole attached to the trunk.

An examination of a transversely cut section of the stem will enable some idea of the arrangement of the tissues to be made out (Fig. 40). In the first place it will be seen that the cortex is surprisingly wide and that the amount of **xylem** is remarkably small. Strong **medullary rays** are a conspicuous

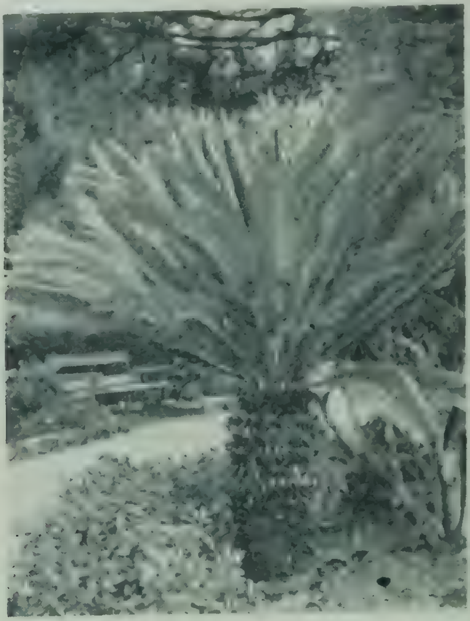

FIG. 39.—*Cycas revoluta.* Habit of plant.

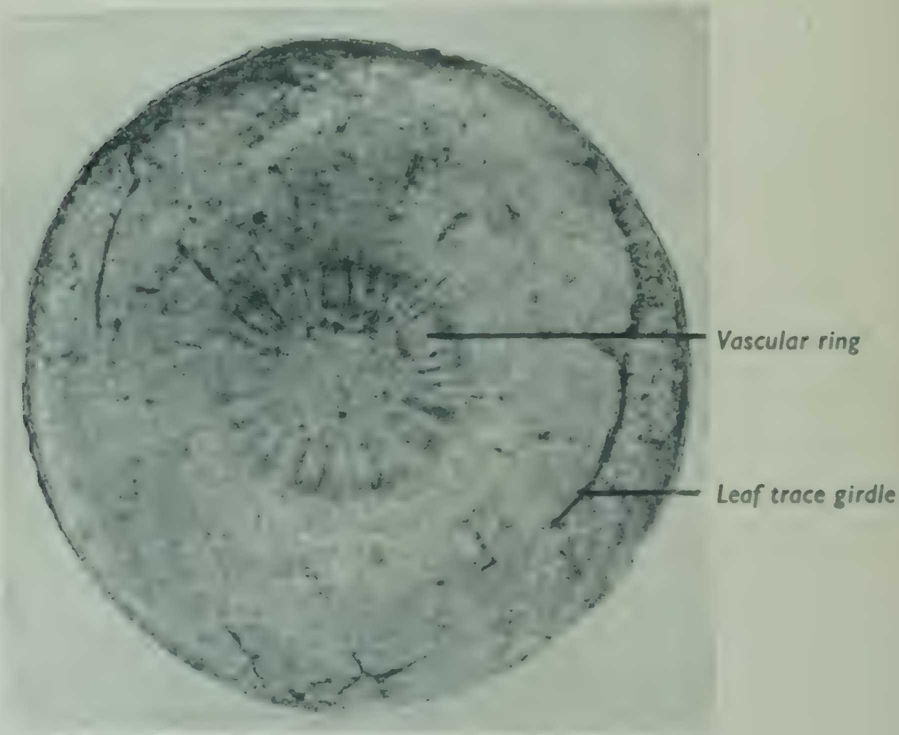

FIG. 40.—*Zamia floridana.* Transverse section of the stem. The anatomy closely resembles that of *Cycas* stem.

feature, but annual rings of secondary wood are not easily made out except in *Cycas* itself, in which successive concentric rings of vascular bundles are formed secondarily. There is a large central **pith.** The leaf traces are given off all round the stele and pass round the circumference before entering the leaf bases. They thus form a conspicuous series of **girdles** in the cortex. The photograph shown in Fig. 39 may be a help to those who have not access to original material.

Transverse Section of the Petiole.—Cut a transverse section through the petiole near the base, stain in Aniline chloride and mount in Glycerine.

The petiole is bounded on the outside by an **epidermis,** composed of rectangular thickened cells, which are covered by a cuticle. Inside are one or two layers of rather thin-walled parenchymatous cells, some containing chloroplasts, which may be assimilatory in function. Internal to this is a wide band of sclerenchyma interspersed with parenchyma. As we pass inwards the central cells become progressively larger, more rounded in shape and less closely packed together. Crystals of Calcium oxalate of various shapes are conspicuous in these large central cells. The **vascular bundles** are arranged in the form of a horseshoe and therefore agree in having the same fundamental grouping as that in the petiole of Ferns. The detail of the vascular bundle is complex. It will also depend on the position of the bundle whether the phloem or xylem lies towards the outside of the section.

In the bundle the most noticeable structure is a large mass of **xylem,** curved on the outside, and coming to a point towards the inside of the bundle. This is the **centripetal xylem,** made up of metaxylem with scalariform thickening, while at the point on the inner side lies the protoxylem. Surrounding the protoxylem on the inner side is a group of parenchymatous cells, embedded in which is a much smaller amount of **centrifugal xylem** which alone corresponds with that in an Angiosperm stem. Inside the parenchyma lies an arc of very small rectangular cells which is the **cambium.** This cambium forms the base of a second arc of tissue which consists of **phloem** mostly derived from the activity of the cambium. Around the periphery of the arc of phloem may be seen the remains of the **protophloem,** which has become crushed, and appears as a darkly staining band of thick structureless material. Outside this there is a single layer of rounded and lignified cells, the **endodermis,** which separates the bundle from the cortex. Each bundle is therefore actually a separate meristele.

Scattered here and there in the centre of the petiole are ducts, round in section, and bounded by a ring of smaller cells, which are the **mucilage ducts.** These run, without branching, up through the petiole into the leaflets.

Transverse Section of the Leaf.—Cut a transverse section through a portion of a leaflet, passing through the midrib ; stain in Aniline chloride and mount in Glycerine.

The lamina will be seen to consist of a thickened **epidermis,** covered by a cuticle on the upper side, followed by a second layer of polygonal cells which may be regarded as a **hypodermis.** Below this is a single layer of elongated **palisade cells** containing chloroplasts. These are followed by a **spongy tissue,** which has large intercellular spaces towards its lower surface, while in the middle the mesophyll is traversed by horizontally running elements, with here and there a mucilage duct. These long cells connect the single midrib bundle to the edges of the leaf, and are generally regarded as a form of **transfusion tissue.** The lower epidermis, like the upper, is made up of rectangular cells strongly thickened and covered externally by a cuticle. Stomata may appear here and there along the lower surface. They are deeply sunken, so that a cavity is formed outside the stomatal opening, which is protected by a pair of guard cells. The pore leads directly into an intercellular space.

In the region of the central vascular bundle the palisade structure persists across and above the bundle, and is accompanied by a parenchymatous tissue containing scattered thickened cells, full of crystals. Below the bundle the mesophyll is replaced by a parenchymatous tissue, many of whose cells, especially near the lower surface, are strongly thickened. The central bundle is surrounded by an **endodermis,** whose cells are oval in section, with thickening on the radial and inner tangential walls. The stele itself is also surrounded by a **pericycle** and has a central mass of **centripetal xylem,** with the protoxylem towards the lower surface of the leaf. It is surrounded by parenchyma, which separates it from a V-shaped group of **centrifugal xylem** elements which lie immediately in contact with the cambium. Towards the lower surface lies the **phloem,** consisting of secondary phloem derived from the cambium and compressed primary phloem, no longer recognizable as such. This latter tissue lies immediately in contact with the pericycle.

> *Transverse Section of the Root.*—Cut a transverse section of the root, stain in Aniline chloride and mount in Glycerine.

The youngest roots are bounded on the outside by a piliferous layer, which in the older root disappears, and is replaced by the thickened outer layers of the cortex. There is a wide **cortex** composed of rounded parenchymatous cells with **mucilage ducts** scattered among them. The **endodermis** is not clearly differentiated, but below this is the **pericycle,** which develops a **pericyclic cambium,** cutting off secondary cortical cells. The central stele is diarch, with two **protoxylem** groups connected by a thin strand, sometimes only one cell wide, composed of **metaxylem.** On either side of the xylem lies the **cambium,** which may or may not have produced any **secondary xylem.** If it has, the small quantity of **secondary phloem** will lie between the cambium and the **primary phloem,** which forms a pair of arcs on either side of the xylem plate.

If the section of the root has been taken from one which has been growing near the surface of the soil and exhibited a somewhat " coralloid " appearance, it will be found that there is a ring of large specialized cells in the middle of the cortex. These cells are thin-walled, their straight radial walls looking like the spokes of a wheel. These cells contain Cyanophyceae, algal filaments belonging to the species *Anabaena cycadacearum.* In a good section it may be possible to see the morphology of these short chains of rather specialized cells, containing the bluish-green pigment, which is characteristic of this group of Algae. The function, if any, of these endophytes is uncertain.

Male Cone.—Examine a complete male cone of this or other species of the Cycadaceae. They are all essentially similar in structure (Fig. 41).

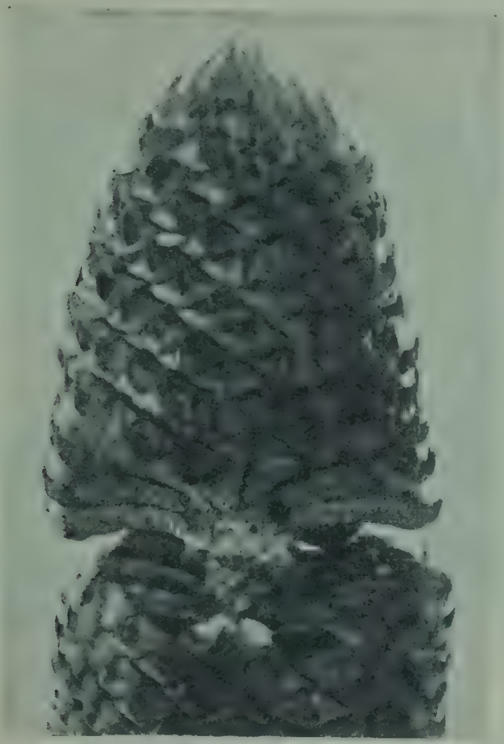

FIG. 41.—*Macrozamia miquelii.* Upper part of male cone.

The cone is long and tapering, not round like the female strobilus. It consists of a central woody **axis,** 12 to 24 in. long, from which the **sporophylls** are given off spirally. Each sporophyll is attached by the narrow end of a flattened triangular stalk which terminates in a flat shield-like extremity with an upturned tip.

On the lower surface of the flat stalk-like part of the sporophyll are large numbers of **microsporangia** grouped together in an irregular manner.

Cut a transverse section across the sporophyll to include a number of these microsporangia and examine. The sporangia are roughly spherical in shape, comparatively large and rather flattened at the top. The wall is composed of a layer of small cells whose radial and inner tangential walls are strongly thickened. The remains of an inner **tapetal layer** tissue may also be found. The **microspores** are small spherical bodies with thick rugose walls.

The sporophyll itself is composed of rounded mesophyll-like cells with intercellular spaces. Many mucilage ducts can be made out, together with scattered vascular bundles, many of which will be cut obliquely. There is a somewhat thickened epidermis around the outside. The sporangia are attached to the sporophyll by short stalks.

Female Sporophyll.—*Cycas revoluta* differs from other examples in that no true female cones are produced, but the apex of the shoot produces alternately spiral whorls of sterile and of fertile leaves (Fig. 42).

The **female sporophylls** are about a foot in length, the lower half consisting of a woody petiole on which are borne about half a dozen yellow or orange **ovules** which arise alternately on each side of the petiole. The upper part of the sporophyll is leaf-like and consists of a number of reduced leaflets which, though brown in colour, resemble on a rather smaller scale the leaflets of the foliage leaves (Fig. 43). These very leaf-like sporophylls, so loosely aggregated at the stem apex, are generally regarded as primitive and as illustrating an evolutionary connection between leaves and sporophylls.

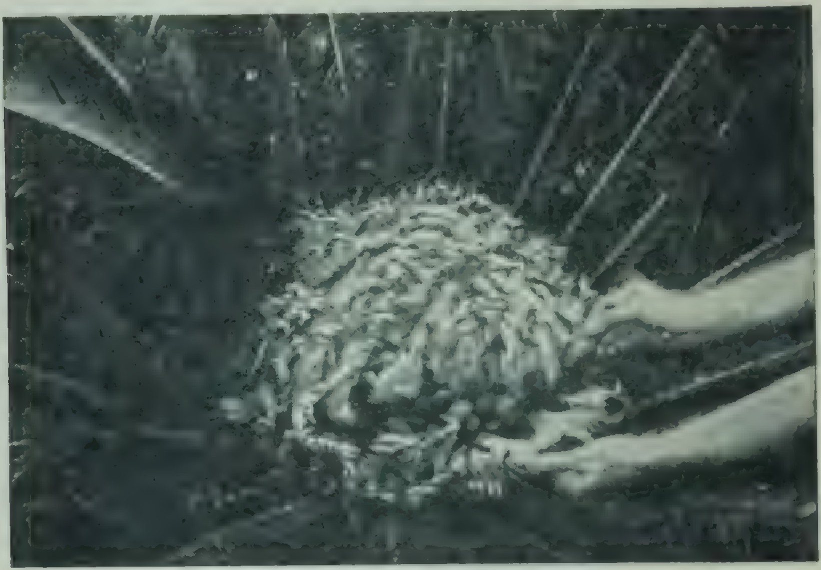

FIG. 42.—*Cycas revoluta.* Strobilus of megasporophylls.
(*From the "National Geographic Magazine."*)

In *Cycas circinalis*, as in the other genera of the Cycadaceae, the female sporophylls are aggregated into compact cones which may be of variable shape and of large size, up to 2 or 3 ft. in length, and weighing many pounds. In such cones the **sporophylls** are compacted together and overlap one another, each being a relatively short and woody structure, consisting of a basal portion which is wide and grows out more or less at right angles to the central axis, and a triangular vertical part which turns upwards to overlap the sporophyll above.

In some genera the female cones are nearly spherical, *e.g.*, *Dioon.*, and the distal part of the sporophyll more leaf-like, but in most they are conical with a massive central axis on which the sporophylls are spirally arranged.

Each sporophyll bears two **ovules** which are attached right and left of the sporophyll stalk with their micropyles inwards towards the cone axis.

> *Ovule.*—The amount of detail which can be made out from a longitudinal section through the ovule will depend largely upon the age and the extent to which the cutting of thin sections has been possible.

The **seed** is an oval body sometimes with a small point at the distal end. Here the remains of the **micropyle** can be seen. The wall of the seed is made up of a stony layer which is really the inner integument. Outside this is the thick fleshy integument which forms the outer covering of the ovule. Inside lie the two straight cotyledons with the root apex of the embryo directed to the top. The embryo lies embedded in **endosperm** which has been derived from the female gametophyte.

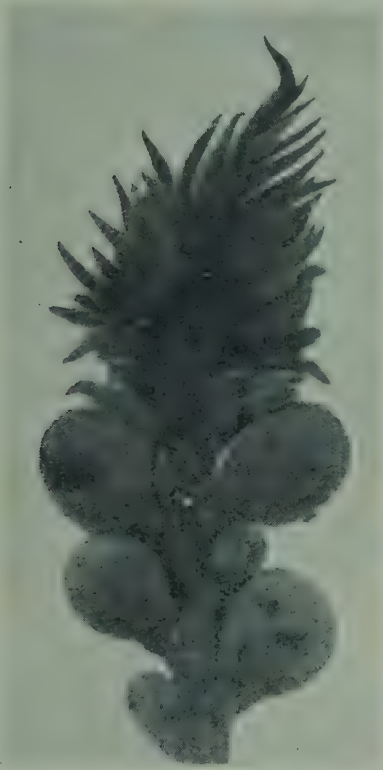

FIG. 43.—*Cycas revoluta.* Single megasporophyll.

Where possible, microtome sections made from specially preserved material should be examined, showing the development of the ovule. Since, however, it will depend upon the particular age of the material what structures are visible, it is impossible to describe such sections in detail here. Students are therefore advised to consult one of the theoretical works [1] on the group for the elucidation of such sections.

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[1] *e.g.*, "Textbook of Theoretical Botany," vol. i. McLean and Cook. Longmans, Green & Co. Ltd.

In a really young ovule it should, in any case, be possible to see the large **female gametophyte** lying in the centre, with two large **archegonia** at its apex. The apex of the **nucellus** should also show the breakdown of cells to form the **pollen chamber.** See Fig. 44.

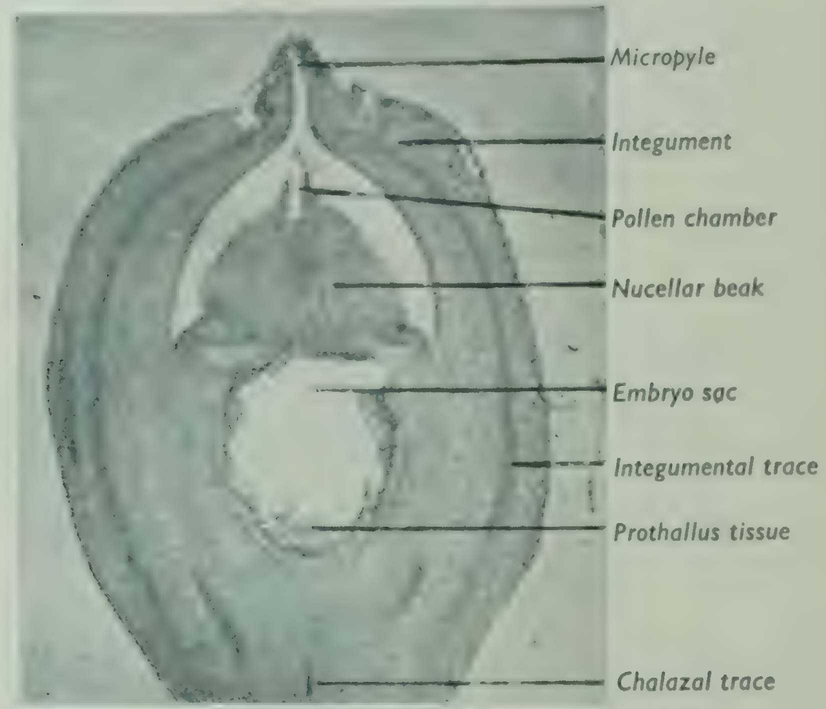

FIG. 44.—*Stangeria paradoxa.* Vertical section of the young ovule in an early stage of the development of the female gametophyte.

4. ***Ginkgo biloba.*** *External Morphology.*—Examine young, leafy branches of the plant.

The **leaves** are fan-shaped, with more or less parallel, dichotomous **veins.** At the base the **lamina** narrows into a **petiole** of a length about equal to that of the lamina. Each leaf lamina shows, normally, one large division splitting it into two approximately equal lobes and there may be other minor divisions. Small leaves are often undivided. The leaves on older stems arise spirally on dwarf **foliar spurs,** which may sometimes be so short that the leaves appear to be in fascicles. On the shoots of the current year the leaves are directly inserted on the main elongating stem, and the dwarf spurs are built up from the axillary buds of these leaves in the second and subsequent years, and may persist for many years thereafter.

Transverse Section of the Stem.—Cut a transverse section of a piece of the current year's stem, stain in Aniline chloride and mount in Glycerine.

The **epidermis** is highly cuticularized and is supported beneath by a sclerotic **hypodermis,** one to three cells thick. Inside this arises the **phellogen,** at an early stage, from the outermost cells. The **cortex** is broad and contains numerous brown **tannin cells,** many cluster crystals of Calcium oxalate and three or four large **mucilage canals,** each surrounded by a sheath of thickened cells. The inner cortex contains tangential rows of **stone cells** with very thick walls and numerous fine **pit canals.** Inside this, and possibly included in the phloem, lies a zone of thick-walled cells without pits. This is separated from the zone of stone cells by a layer of parenchyma, which may be the equivalent of the **endodermis.** The **phloem** and **cambium** are very like those of *Pinus.* The **xylem** forms a continuous zone in which the position of the primary bundles is only distinguishable by the **protoxylems,** which are numerous. The **medullary rays** are all narrow and uniseriate. In the **medulla** more mucilage canals may occur.

In older stems the phloem is broad and shows tangential layering of alternating rows of large sieve tubes and crushed phloem parenchyma.

> *Longitudinal Section of the Woody Stem.*—Cut radial and tangential sections of the woody zone, stain in Aniline chloride and mount in Glycerine.

The radial section will show the numerous narrow, spiral elements of the **protoxylem.** The younger wood shows **bordered pits** in the radial walls, and between these pits **rims of Sanio** are often clearly visible. Many tracheids bear only one loose row of pits. Annual rings may be seen, marked by the narrower tracheids formed in autumn. The **medullary rays** are seen to be very short in the vertical direction.

In the tangential section it will be seen that many of the medullary rays are only one cell high and that few are more than three cells high. In the dwarf spur shoots they are considerably longer.

> *Transverse Section of the Petiole.*—Cut a transverse section, stain in Aniline chloride and mount in Glycerine.

The general appearance recalls a needle-leaf of *Pinus.* The section is semicircular, the flattened side being adaxial. Several long **mucilage canals** occur in the **cortex** and there are scattered groups of **hypodermal fibres.** There are two **vascular bundles,** tilted at an angle to each other and surrounded by a common investment of thickened cells which may represent an **endodermis,** as between it and the bundles lies a zone of parenchyma cells in the position of a **pericycle.** The bundles are collateral, with the **protoxylems** turned towards the adaxial side, *i.e.*, endarch.

> *Transverse Section of the Leaf.*—Cut a transverse section of a portion of the leaf lamina at right angles to the veins, stain in Aniline chloride and mount in Glycerine.

The **upper epidermis** is not very highly thickened ; below it is a **palisade layer** of irregularly lobed cells, which are frequently branched at their lower ends, where they are in contact with the **spongy mesophyll.** The cells of the latter tissue are transversely elongated, with lobed and irregular outlines. Among them are numerous round **idioblasts** containing large aggregates of Calcium oxalate crystals. The **lower epidermis** consists of small thick-walled cells, among which are sunken **stomata.** Here and there may also be seen sections of the large **mucilage canals,** lined by a delicate **epithelium** and surrounded by a sheath of cells containing tannin.

The **vascular bundles** are simple and collateral. The **phloem** is flanked on each side by cells containing dense protein reserves and the **xylem** is also flanked by round, empty cells with reticulate wall thickenings, the **transfusion tissue.** A few sclerenchyma cells are generally present on the adaxial side.

Reproduction : The Male Strobilus. General Morphology.—The male catkins are about 1 in. long, with a delicate axis, bearing **sporophylls** in spiral order. Each sporophyll has a slender stalk and bears terminally two pendent, slightly curved **microsporangia** (pollen sacs). Where these are attached to the stalk there is also a small upwardly pointing apiculus, spoken of as the " hump." In section it will be seen to contain a large **mucilage cavity**.

Male Strobilus. Transverse Section.—Cut transverse sections through the middle of a male catkin and mount the pieces in Glycerine. Microtome sections, which will hold the parts in their proper positions, are preferable.

The axis shows a ring of small **vascular bundles,** surrounded by a partial **endodermis.** The **cortex** and **medulla** are lacunar. From the axis arise the stalks of the **sporophylls,** each containing a single vascular bundle, like those in the leaf, with **transfusion tissue.** The walls of the **sporangia** are three or four cells thick and dehisce along the sides next to each other. The **microspores** are small and smooth-walled, with two little projections like miniature wings. The cells of the sporangial walls show cuticularized ribbing, like the middle layer of an angiospermic anther wall, but only on the dehiscing side of the sporangium.

Reproduction : The Female Strobilus. General Morphology.—The **ovules** are placed in pairs at the top of stalks or **peduncles** about 1½ in. long, which arise in clusters on the foliar spurs. A striking feature of the ovule is the **collar**, a basal ring of tissue surrounding the ovule. It is specially conspicuous in the young stages, as it does not enlarge with the growth of the seed.

Transverse Section of the Peduncle.—Cut a transverse section through an ovular peduncle, stain in Aniline chloride and mount in Glycerine.

The anatomy of the peduncle corresponds closely to that of the petiole, except that it has **four vascular bundles** instead of two. This has been interpreted as an indication that the peduncle is formed from two petioles adherent by their adaxial faces.

Longitudinal Section of the Ovule.—Cut a median longitudinal section through the pair of ovules at the top of a peduncle ; mount in Glycerine.

The vascular bundles of the peduncle end, upwards, is an extensive tissue of short, spiral tracheids with which the collar is liberally supplied. A protoxylem trace approaches the base of each ovule, but ends near the base of the nucellus. The ovule itself consists of a massive **integument,** in which there is no vascular tissue, opening at the apex by a prominent **micropyle.** The **nucellus** is free from the integument to its base. At the apex is a large **pollen chamber** excavated from the nucellar tissue, and having a long **nucellar beak** which goes about half-way up the micropyle. Near the bottom of the nucellus is a large **embryo sac** which will probably show one of the stages of prothallus formation. If the prothallus formation is nearly complete, a pair of large **archegonia,** short and unusually broad, may be seen at the apex of the embryo sac. That the enlargement of the embryo sac produces considerable downward pressure may be seen from the layers of flattened cells which usually underlie it.

5. ***Ephedra.*** *External Morphology.*—Examine a fresh or herbarium specimen of a shoot of any cultivated species of *Ephedra.* All the species are similar vegetatively.

The main stems are woody and markedly jointed. Each node bears a pair of **scale leaves** whose bases are **connate** around the stem. At each node in the upper part of the plant there arises a group of six or eight slender green branches, originating in two clusters from multiple buds in the axils of the two scale leaves. These branches are green and assimilatory. Their internodes are long and at each node there is a pair of smaller scale leaves which are **decussate,** *i.e.*, each pair is at right angles to those at the nodes above and below. These branches disarticulate readily at their nodes, especially in winter, and give rise to fresh side shoots from the remaining nodes in the following year.

Ephedra suckers readily from the roots, and in time forms large, spreading clumps.

Transverse Section of Stem.—Cut a transverse section through one of the lower internodes of an assimilatory branch, stain in Aniline chloride and mount in Glycerine.

The general distribution of tissues follows essentially the dicotyledonous type. The **epidermis** is strongly cuticularized and shows numerous **stomata,** each sunk at the bottom of a deep, narrow pit. The **outer cortex** consists of palisade-like cells, rich in chloroplasts. Among these cells are numerous radial groups of **sclerenchyma.** The **inner cortex** is parenchymatous. There is no visible endodermis or pericycle. A single ring of simple, collateral **primary bundles** surrounds the broad **medulla.** Each bundle has a prominent **cambium,** and, in older internodes, this extends across between the bundles and shows the beginning of **secondary thickening.** One of the most noteworthy points is the presence in the xylem of **vessels,** of larger diameter than the tracheids, which is an almost unique feature in a Gymnosperm. A few thick-walled sclerenchyma fibres occur outside each primary phloem group and may possibly represent a pericycle.

The Male Strobilus.—*Ephedra* is normally dioecious, so that only strobili of one sort will be found on a single plant.

Each **male strobilus** stands in the axil of a scale leaf. The strobilar axis bears a number of thick, closely set **bracts** arranged in pairs, decussately. In the axils of the bracts are the **male flowers.** In some species all the bracts are fertile, but this is variable. Each male flower has a **perianth,** consisting of two scales, from between which rises the single stamen, bearing a variable number of **anthers.** The anthers may be either sessile or each may have an individual stalk. The anthers dehisce by an apical slit, releasing the elliptical **pollen grains.**

The Female Strobilus and Ovule.—These occur in the leaf axils, in the same way as the males. They are usually somewhat shorter than the males, with only usually two to four pairs of bracts. Cut longitudinal sections through the strobilus and select those which show ovules; mount in Glycerine.

The **ovules,** each of which represents a **female flower,** are terminal on the strobilus. In some species there are only two, which appear to be axillary to the uppermost bracts, but in others there may be only one flower or, alternatively, a group of three or four, surrounded by a whorl of several bracts, as if several nodes had coalesced. At the base of the strobilus there may sometimes be numerous pairs of small scales, which may be either regarded as reduced sterile bracts or as the scale leaves of a supporting branch.

Each ovule has two **integuments.** The outer integument is formed of four united segments and probably represents a **perianth.** The inner integument consists of two united segments and is prolonged upwards into a long, curled or twisted, **micropylar tube.** The **nucellus** is free from the integument only in its upper half. There is a large apical hollow, or **pollen chamber,** and the **female prothallus** occupies the greater part of the nucellus. The lower part of its tissue is much denser than the upper part, in which a pair of long, narrow **archegonia** may be seen, rather deeply sunk in the prothallus, below the pollen chamber.

PART FOUR

MORPHOLOGY AND HISTOLOGY OF ANGIOSPERMS

Section 1. Stems and their Modifications

A. EXTERNAL MORPHOLOGY OF STEMS

1. THE NORMAL STEM

Vicia faba (Broad Bean).—The shoot may be regarded as a good type of **herbaceous stem.** There is an obvious longitudinal division of the stem into segments, the joints between which are called **nodes** and the lengths of stem between the **internodes.** The lengths of the internodes vary, being short at the base and apex and longest in the median portion of the axis. Leaves are borne only at the nodes, and the angle between leaf and stem is called the **leaf axil.** In the axils are formed **lateral** or **axillary buds** which develop into flowering branches.

Each leaf is compound, *i.e.*, it has more than one leaf blade or **lamina,** and there is a **petiole** or leaf stalk, the tip of which is prolonged between the leaflets as a rudimentary third leaflet. Where the petiole joins the stem there are two small green leaflets called the **stipules,** one on each side of the petiole. These are protective organs for the young leaf, and they reach their full size while the leaf is still quite small and is enclosed between them.

The lowest node or two on the stem do not develop normal leaves but rudimentary structures composed of the stipules united together. The lowest node of all is that of the **cotyledons**—large solid structures which remain enclosed within the seed coat. They are modified leaves, although white and quite unlike normal leaves in appearance, and they have dormant buds in their axils.

The apex of the shoot is hidden in a cluster of young folded leaves, the **apical bud.** For a short period in the development of each leaf it remains folded upwards, protecting the apex of the stem, but eventually it expands, leaving the apex covered by still younger leaves which expand in their turn. These leaves are all developed from the **growing point** of the stem which is the actual apex. It can only be seen if the apical bud is cut through longitudinally with a razor (from below upwards), when it will be revealed as a rounded colourless structure closely enveloped in minute embryonic leaves and possibly flower buds, axillary to the leaves.

See also, for root system of Bean, page 213.

Acer pseudo-platanus (Sycamore).—The woody shoots may be examined as a contrasting type to the herbaceous shoot of the Broad Bean. The bark of the younger portions is smooth and greenish and marked with many brown **lenticels,** while the older bark becomes rough and fissured.

Each branch has an **apical bud** with smooth green **bud scales** arranged in alternate pairs (decussate). The successive positions of the apical bud at the end of former growing seasons can be seen by the narrow zones of closely set transverse lines which occur every 3 to 6 in. on the stem. These lines are the scars left by the scales of former winter buds when they dropped off as the bud began to grow in spring. Leaves develop at each **node** on the shoots of the current year's growth, and in winter, when they have dropped, their places are marked by curved **leaf scars** of a pale colour, on which may be seen the three brown dots that mark the positions of the **leaf trace bundles,** which are vascular strands running from the stem to the leaf.

Each leaf axil produces an **axillary bud** which may give rise to a new branch. The pair of buds at the base of each year's growth remain dormant, however, and are gradually covered over by the growth of bark as the shoot gets older. These dormant, concealed buds retain their vitality for many years and may break out into active growth from quite old branches, or even from the trunk itself if the tree is cut back.

Pyrus malus (Apple).—In many fruit trees there is a distinction between the **long shoots,** which bear only leaves, and the short lateral shoots or **spurs** on which the fruit is formed. In the Apple the long shoots have smooth bark with pale lenticels and internodes 1 to 3 in. long. The spurs, on the other hand, grow only a few millimetres per annum and are marked almost all along by the transverse scars of bud scales. The terminal bud of the spur is transformed into an inflorescence and thereafter one or two lateral buds, on the spur, develop each year into fresh inflorescences **(sympodial growth).**

The fate of any given axillary bud on the stem is not fixed, and it depends largely on the pruning of the tree whether it develops into a long shoot or into a spur.

Prunus cerasus (Cherry).—This also shows long and spur shoot growths very well. The bark of long shoots is brown when young but becomes striated and silvery with age.

The spurs are **monopodial,** that is, they keep a terminal bud, and the flowers develop from the laterals. A cluster of flowering buds usually also develops at the base of each year's growth on the long shoots, growing from the axils of last year's bud scales. Flowering buds are usually stouter and more obtuse than the leaf buds.

Prunus domestica (Plum).—Like the Apple and the Cherry, the Plum fruits on spurs, but the spurs are longer than is usual in the other cases, and are studded with swollen **leaf bases.** The spur is again **monopodial,** and the terminal bud is small and sharp pointed.

2. MODIFIED STEMS

(*a*) **Rhizomes.**—Horizontal underground stems bearing scale leaves and producing leafy aerial shoots and adventitious roots.

Agropyron repens (Couch-grass).—A young plant of Couch-grass shows numerous lateral shoots (tillers) which arise in the axils of the lowermost leaf sheaths. Some of these are **decumbent,** that is to say, that after a horizontal growth of one or two internodes they turn upwards and cease to elongate, forming instead an apical cluster of the broad flattened leaves which are characteristic of the aerial parts. Other shoots retain their recumbent growth for some time and penetrate the soil for considerable distances, forming **rhizomes,** with internodes 1 to 3 in. long, and with white, pointed **scale leaves.** These rhizomes produce from their nodes **adventitious roots** which break out through the bases of the scale leaves, and also branches, both decumbent and rhizomatous, so that they are active agents in spreading the plant vegetatively and make it a very difficult weed to eradicate. The physiological reactions which determine the character of any one lateral shoot are not understood. Depth below the soil surface may be responsible but internal controls are probably concerned.

Ammophila arenaria (Marram Grass).—This is the grass which covers sand dunes. The **rhizomes** grow for long distances below the sand, and are the means enabling *Ammophila* to cope with the dangerous mobility of its substratum.

The internodes of the rhizomes are several inches long. At each node is a cluster of **adventitious roots** which have a white corky covering, and a sharp-pointed scale leaf, usually dry and tattered. The rhizome ends in a long sharp-pointed **apical bud** well shaped for boring through the sand. This bud eventually turns up and becomes a new, leafy, flowering shoot above ground. The rhizome is sympodial, its growth being carried on in successive seasons by the development of axillary buds.

Tussilago farfara (Coltsfoot).—The **rhizome** is of a semi-fleshy type, but nevertheless it grows very fast, and the plant spreads aggressively where established. Internodes are often more than 1 ft. long. The nodes bear scale leaves, except at the apices, which turn up and bear a spiral succession of foliage leaves. **Adventitious roots** may be formed anywhere, and branching is sympodial.

Polygonatum multiflorum (Solomon's Seal).—The underground part consists of a hard white **rhizome,** up to a foot in length, decaying in its older parts and terminating apically in a bud which produces the next year's flowering shoot. Each of these aerial shoots is terminal and temporary, and therefore closes the growth of the rhizome. When it drops off a scar is left on the rhizome which has fancifully been compared to the magical *Sigillum Solomonis*.

The rhizome is marked transversely with the lines (nodes) of attachment of **scale leaves.** Each year an axillary bud from one of these nodes develops either into a new terminal portion of the rhizome, which is thus **sympodial,** or else into a lateral branch.

Adventitious roots are freely produced all over the rhizome. This is normal for most Monocotyledons.

Iris germanica or other species. The **rhizome** here is even thicker than in *Polygonatum*, and is filled with **reserve starch** from which Orris powder, the basis of some face-powders, is obtained. There are no long internodes, each fleshy leaf base lying close against the next, so that their scars, when the leaves have gone, are contiguous. Branching is again sympodial, the lateral buds from which new branches develop, alternating on opposite sides of the rhizome, and the fleshy **adventitious roots** are produced endogenously from below the younger part of the rhizome.

Convallaria majalis (Lily of the Valley).—Like Coltsfoot, this plant produces a semi-fleshy **rhizome,** more adapted for spreading than for storage ; the older portions are hard and woody. There is a large **apical bud** on each branch, which turns up into a leafy flowering shoot, and further growth of the rhizome is therefore from axillary buds (sympodial). During the season these buds expand, forming branches 2 to 3 in. long, each with several nodes marked by scale leaves and bearing abundant fleshy **adventitious roots.** The apical portion of each then forms a well-protected pointed **winter bud** which grows up through the soil, coming above the surface in spring and producing foliage leaves and an inflorescence. The number of flowering shoots formed thus increases every year.

(*b*) **Tubers.**—Short swollen stems bearing buds, formed either on the main stem or on branches, usually underground.

Solanum tuberosum (Potato).—At one end of the tuber is a small point or tail marking the point of attachment of the underground branch on which the potato grew. At the other end is a group of small pits, the " eyes," in which are minute **dormant buds.** On one side of each eye is a slight ridge, which marks the position of the **scale leaf** in whose axil the eye bud arises. These scales themselves can only be seen when the Potato is very small, as they soon drop off.

In some Potato plants, especially if insufficiently earthed up, tubers are found above ground in the axils of the lowest leaves. On these the scale leaves persist and become green leaflets.

Helianthus tuberosus (Jerusalem Artichoke).—The tubers show their stem character more evidently than do those of the Potato, for they are often more or less elongated and irregularly branched. There are numerous visible **buds,** and each branch (when any are formed) ends in a large pointed bud with conspicuous **bud scales.** The presence of such scales, which are reduced leaf structures, is definitely characteristic of stems as contrasted with true roots.

(*c*) **Corms.**—Solid underground main stems in the form of cushions with scale leaves.

Crocus or ***Gladiolus*** (any garden form will do).—The stem, as in many Monocotyledons, is subterranean and takes the form of a rounded cushion-like structure called a **corm.** Cut one of these in half longitudinally and it will be seen to be solid stem tissue (mostly **cortex**) with a well-marked central **stele** running from the base, where **adventitious roots** are formed, to the **apical bud,** which develops into the foliar stem. Test the cut surface with Iodine. It will show a strong starch reaction (see p. 49). The outside of the corm is covered with loose, dry **leaf scales.** Strip these off and they will be seen to arise from **nodes,** which show as dark lines running horizontally. At certain points on these nodes are **axillary buds,** partly sunken in the corm tissue. From these, new corms usually develop but one or more of them, at the nodes nearest the apex, develop instead into new foliar and flowering shoots to replace that of the current year, which lasts only for one flowering season.

The buds are connected by branch steles to the main central stele.

(*d*) **Bulbs.**—Underground stems in the form of buds, with relatively large fleshy scales.

Tulipa (Garden Tulip).—The true stem is the underground **bulb.** Cut this longitudinally from below upwards. The main portion of the bulb consists of fleshy **leaf bases** which arise from a very small button-like **stem** which forms only the base of the bulb. These leaf bases are really tubular, as may be seen in cross-section, and they and the stem are stored with reserve starch. In the axils of the leaves there are small **axillary buds** which develop new bulbs (" offsets "), thus multiplying the plant. Some other bulbs, *e.g.*, *Narcissus*, do this even more freely than the Tulip. Because the leaf scales completely surround the bulb, this type of bulb is called **tunicate.**

In the centre of the bulb will be seen the **apical bud** which produces the green leaves and flowering shoot. At the base of this bud is another smaller one, really an axillary bud of the innermost bulb scale, which will replace the flowering bud in the next season.

The flowering bud is completely developed in its parts by autumn, and is ready for rapid growth by simple expansion in the spring.

(*e*) **Stolons.**—Horizontal underground branches from an aerial main stem.

Mentha spicata (Mint).—The **stolons** are creeping branches of square section resembling the aerial stems, except for the absence of chlorophyll and the reduction of the leaves to scales, which soon vanish, and the production of adventitious roots at the nodes. These stolons come from axillary buds at the base of each upright stem. They grow monopodially, eventually turning up at the apex and forming new leafy shoots. They also produce lateral axillary branches from their underground portions.

In some cases these stolons form above ground, and should then be classed as runners.

Alternatives: Perennial Sunflower (*Helianthus rigidus*) or Michaelmas Daisy (*Aster* sp.).

(*f*) **Runners.**—Horizontal branches formed on the soil surface.

Potentilla reptans (Tormentil).—Common in every hedgerow and in most gardens. The parent plant consists of a tuft of leaves arising from a contracted stem. Thin runners, with 2 to 6 in. internodes, arise from the leaf axils and grow in all directions. At each node a new tuft of leaves may be formed on a contracted axillary branch, the runner continuing onwards, while from the axils of the branch leaves new runners may arise. The runner apex may itself be transformed into a leaf-bearing tuft, with new runners arising from its leaf axils. Alternatively a pair of opposite leaves may be formed at a node, each with an axillary runner, between which the apex of the original runner aborts.

The result, in any case, is a widely spreading and complex system of vegetative propagation. Try to identify cases such as the above in your material. In addition, note that sometimes the original runner, sometimes the side shoot and sometimes the secondary runners, may be the most developed, and that this varies a great deal. There are also frequently adventitious roots at the nodes.

Alternatives: *Potentilla anserina*, *Mentha* species, and *Fragaria vesca* (Strawberry), all develop good runners which are either simple or form only single sympodial branches.

(*g*) **Cladodes.**—Branches flattened or compressed, assuming the form and functions of leaves.

Ruscus aculeatus (Butcher's Broom).—The structures which appear to be the leaves are really **cladodes** or flattened branches, their true nature being shown by the fact that they arise in the axils of small blackish **scales,** which are the true leaves very much reduced. Furthermore, each cladode bears, in the middle of its upper (adaxial) surface, a dwarf branch, also subtended by two scale leaves on which the unisexual flowers are formed.

The anatomical structure of a cladode is what one would expect in a stem pressed flat, rather than that of a true leaf. Each cladode ends in a sharp spine.

Asparagus plumosus (or other species). The **scale leaves** are modified into small deflexed spines, each with two pairs of minute **stipules** or, on the smallest branches, with two stipules only. In their axils the needle-like **cladodes** arise in **fascicles** or bundles of about eight to fifteen, growing from two whitish tubercles which are attached side by side on the stem.

(*h*) **Thorns.**—Branches of limited growth, ending in a sharp woody point.

Ulex europaeus (Gorse).—All the branches end in spiny points instead of in buds. The **thorns** are short branches, arising from the axils of triangular green **scales,** which are the true leaves. Each thorn is **compound** and itself bears scale leaves, from the axils of which arise small secondary thorns. These latter often bear two minute scale leaves on opposite sides of their bases, and in the axils of these arise tertiary thorns about 5 mm. long. In some cases even quaternary thorns may be found.

Crataegus oxyacanthoides (Hawthorn).—The thorns are developed from axillary buds which begin growth as normal side shoots. After growing for an inch or two, however, the shoot apex becomes lignified, the young leaves surrounding it drop off and a sharp point is left. Lateral buds on the thorn itself may, however, continue for a short time in growth, and produce either branches of secondary rank or flowers.

(*i*) **Other Modifications.**

Brassica oleracea var. capitata (Cabbage).—The plant is biennial, that is, it forms only a vegetative shoot in the first season, while the flowering shoots develop in its second year of growth. The first season's stem is short and consists of little but an enormous **apical bud,** the " head," the leaves of which remain wrapped together much longer than is usual.

Brassica oleracea var. gemmifera (Brussels Sprout).—See page 197.

Brassica oleracea var. botrytis (Cauliflower).—This is distinguished by its **abortive inflorescence** or flower head. Enormous numbers of flower buds are formed, but the flower stalks, instead of developing normally, remain short and fleshy and the crowded buds are partly or completely fused together into irregular masses. A few, however, may eventually develop into perfect flowers.

Daucus carota (Carrot).—The plant is biennial. In its first year of growth the **tap root** is formed, which consists partly of the true main root and partly of the **hypocotyl** (see p. 261). The true stem is not more than ½ in. in length. Strip the leaves from a young carrot plant and the stem will be seen as a short, green, conical structure ending in a small bud. In such a stem the internodes are so much contracted that the leaves from successive nodes are in contact at their bases, and appear as if arising from the top of the root (**radical leaves**). In the second year the apical bud grows into the flowering stem with normally elongated internodes.

Alternatives : Parsnip (*Pastinaca sativa*), Plantain (*Plantago lanceolata*).

Brassica napus (Turnip).—Cut a young turnip in half transversely. A narrow **cortex** will be seen surrounding a thin ring of **vascular tissue.**

There are also numerous vascular bundles (**medullary bundles**) scattered all through the central **pith** or **medulla.** Now cut a slice across the top just below the leaves. There are no medullary bundles at this level, but there is a much thicker ring of vascular tissue, and there are numerous isolated phloem bundles in the cortex. Cut a series of thin transverse slices from above downwards. A level will be found at which the transition from one type of structure to the other occurs. The alteration is easily seen. This is the base of the true stem, and the point of junction with the tap root.

Sarothamnus scoparius (Broom).—This is a type of plant usually bearing leafless shoots, often called a **"switch plant."** The leaves are produced only for a few weeks in the spring. They are very small and trifoliolate, but, insignificant though they may seem, during their existence photosynthesis is more active than at any other time. When they have been dropped the photosynthetic function is carried on by the stem tissues, which contain a palisade layer, like that normally found in leaves, and stomata which are sunk in " protective " grooves. (See Part VIII, p. 386.)

Broom normally prefers dry sandy soil and is a **xeromorphic** type; that is to say, it exhibits structural features which appear to be concerned with protection against excessive water loss. It is unsafe, however, to accept any structures as truly protective simply by deduction and without experimental proof.

***Opuntia* sp.** (Prickly Pear).—This is a good type of **stem succulent** in which the stem segments (they are *not* single internodes) are flattened, but fleshy in texture, and carry on the assimilation of Carbon dioxide by means of chlorophyll. They thus replace, physiologically, the leaves which are absent. The segments are dotted over with small groups of spines which arise from abortive buds. The position of each of these marks a node. Immediately above each group of spines is a true bud from which a branch or a flower may arise.

3. BRANCHING

Aesculus hippocastanum (Horse Chestnut).—In its young state the tree is **monopodial,** that is, each branch possesses an apical bud which continues growth directly from season to season. When the tree reaches flowering age, however, the terminal buds are transformed into flowering shoots and, when the fruits have ripened, these inflorescences drop off, leaving a scar. Growth is then continued by means of the axillary buds of the uppermost pair of leaves. This replacement process is repeated each year and constitutes **sympodial** branching. Where the two axillary buds develop into two equal branches the arrangement is called **pseudodichotomy.**

Syringa vulgaris (Lilac).—As in Horse Chestnut the inflorescences are usually terminal on the branches, and the subsequent growth is provided for by the top pair of axillary buds which grow larger than the others and form new shoots in the following year. Often these axillary buds are supplemented by others, axillary to their lowest bud scales, so that a group of six or more buds may be formed at the end of the shoot, only one or two of which may develop into flowering shoots, the rest forming leafy branches.

Tilia vulgaris (Lime) or ***Ulmus procera*** (Hedgerow Elm).—The branching is **sympodial,** though superficially it resembles monopodial growth. The apical buds of the shoots are dropped at the end of the growing season and replaced by the uppermost axillary bud, which stands as a rule so close to the true apical bud that it very soon thereafter appears to be terminal. The position of the lost bud can be seen in the winter shoot by the round scar it leaves at the base of the end bud of each shoot.

4. BUD STRUCTURE

Brassica oleracea var. gemmifera (Brussels Sprout).—The " sprouts " are simply very large axillary buds which continue to grow after their subtending leaves have dropped off.

Cut one longitudinally, taking care to pass through the centre. This will show the conical **bud axis** or stem, terminating at the **vegetative point,** which is approximately in the centre of the bud. Round it are closely folded the young leaves, arising in a spiral succession, the smallest and youngest in the centre, nearest to the growing point. Note that the inner leaves form only a yellow pigment, **etiolin,** and that chlorophyll only appears in the leaves near the outside, where light penetrates.

It may be seen, with care, that the leaves of this bud have also their own axillary buds.

> ***Aesculus hippocastanum*** (Horse Chestnut).—Terminal bud of branch, cut longitudinally. Make the cut from below upwards as close to the median plane as possible.

The bud is usually a **flowering bud** (not a *flower* bud), and the growing point of the bud stem is transformed into an **inflorescence,** of which the parts are all preformed in miniature, in the autumn before the year in which the flowers are due to open. This young inflorescence is surrounded and protected by a number of specialized leaves, formed on all winter buds of trees and known as **bud scales.** They have only a small amount of chlorophyll and their outer surfaces are brown and corky. The outer scales secrete **resin,** which gums the scales together and forms an additional protection for the delicate tissues at the centre. Growth of the tree has usually ceased by July, after which the terminal buds assume their winter form.

Forsythia suspensa.—The winter twigs have a **cluster of axillary buds** at each node, usually four to each leaf, of which the uppermost is the longest and becomes the flower in spring. The others are vegetative and form clusters of leaves on suppressed branches. Pruning, however, may cause these branches to develop.

B. PRIMARY HISTOLOGY OF STEMS

1. DICOTYLEDONS

Tropaeolum majus (The Garden Nasturtium, climbing variety).—Transverse section of fully grown internode stained with Aniline chloride.

Externally there is the **epidermis,** its cells having a rough outer surface of **cuticle.** Inside this come three to four layers of **collenchymatous cortex,** with conspicuous white thickening of the cell walls, composed of cellulose. Within this again are two to three layers of thin-walled cortical cells with **chloroplasts.** These layers are bounded inwards by the **endodermis,** a well-marked band of one layer of square-shaped cells, with slightly stained suberized walls.

All the tissues internal to this constitute the **stele.** First comes the **pericycle,** which in this stem is an ill-defined layer, one to four cells thick, of small parenchyma cells. It can be best seen opposite the **medullary rays,** the cells of which, in mature stems, are lignified, while those of the pericycle are not. Each **vascular bundle** consists of a wedge of **primary xylem** with the point of the wedge, the **protoxylem,** directed inwards and marked by small lignified cells, the **spiral vessels.** The **metaxylem** consists of a group of large **vessels,** among which are smaller **tracheids** and **xylem parenchyma** (see longitudinal section). The **phloem** lies externally to the xylem and is composed of thin-walled cells polygonal in section, the largest of which are **sieve tubes.** In these may often be seen transverse, perforated walls, the **sieve plates.** By the side of the sieve tubes are small **companion cells** with dense contents, also **phloem parenchyma.** Between the xylem and phloem is the flat-celled **cambium.**

The centre of the stem is occupied by the large-celled **medulla** or **pith,** from which extend outwards the **medullary rays,** of lignified parenchyma, lying between the vascular bundles.

Tropaeolum majus (The Garden Nasturtium).—Longitudinal radial section (*i.e.*, through the centre) stained with Aniline chloride.

The outer tissues of the cortex show much elongated cells, or **collenchyma,** with thick walls ; the inner cortex is thin-walled, with chloroplasts. **Phloem** and **cambium** are alike thin-walled and are not easy to separate in longitudinal section. The phloem, however, shows good **sieve plates** in the sieve tubes, which must be looked for with the high power. The plates are seen either in section or edgewise and show perforations clearly. All these cells show cytoplasm and nuclei. The **endodermis** cells are strictly rectangular, their walls showing traces of suberization.

The **metaxylem vessels** are very large, thick-walled, and covered with small oval **pits.** The network of delicate longitudinal bars on the vessel walls is formed by the outlines of neighbouring **tracheids.** The vessel elements are short and are marked out by transverse bars at intervals on the vessel walls. Innermost come the **protoxylem** vessels with spiral lignification, the turns of the spiral being loosest on the innermost vessels, *i.e.*, those formed first, as the stem xylem is endarch. The innermost of all have only a very incomplete spiral, the thickening in many cases being only a series of detached rings.

Cucurbita pepo (Vegetable Marrow).—Transverse section stained with Aniline chloride.

The single-layered **epidermis** bears numerous **hairs** of various sizes, each hair consisting of a single row of cells ending in a pointed cell and, except in the case of the largest, all based upon a single epidermal cell. The **cortex** is for the most part only two to four cells wide, except where bands of **collenchyma** occur, formed of small cells with thickened corners. The **pericycle** is easily seen in the form of a band of lignified cells, immediately outside of which is the **endodermis,** a single layer of oval cells, larger than the other layers of the cortex. The stele occupies about nine-tenths of the stem, but the pith disappears in older internodes, leaving a cavity in the centre.

There are two rings of **vascular bundles,** the bundles of the outer ring opposite the ridges of the stem, and the bundles of the inner ring opposite the furrows. In each bundle the **xylem** starts at its inner extremity from several **protoxylems** and develops **metaxylem vessels** of very large size, which is a characteristic of most climbing plants. There are two **phloem** groups to each bundle, one on the outside of the xylem and the other on the inside, the former separated from the xylem by a rather indefinite layer of **cambium.** Such bundles are called **bicollateral.** The phloem is easily distinguished from the cambium by the dense contents of the sieve tubes.

Cucurbito pepo (Vegetable Marrow).—Longitudinal section stained with Aniline chloride.

The cell elements are so large that very interesting details of the vascular structure can be seen. The **endodermis** is easily seen as a single layer of narrow oblong cells with walls of a darker shade than the cortical parenchyma. In the **phloem** the **sieve tubes,** with **sieve plates,** may easily be seen and their protoplasmic contents, with more or less degenerate nuclei, observed. The **companion cells,** with granular contents, are visible, and the very narrow cells of the **cambium** are also well shown. In the **xylem** the largest (newest) vessels come next to the cambium. They have thick transverse ribs at intervals, marking off segments about six times as long as broad. These segments are the original cell elements which compose the vessel. Vessel walls may also show a tracery of ridges which mark the outlines of the neighbouring xylem parenchyma. The sequence of vessels from the cambium inwards towards the pith is as follows :—

(*a*) Large, with oval pits.
(*b*) Medium, with transverse slit-like pits.

(*c*) Medium, with spirals.
(*d*) Small, with spirals.
(*e*) Very small, with isolated rings.

The last two categories make up the **protoxylem.** They are often separated from the rest by rows of parenchyma cells. Besides the vessels one may note also many **tracheids** with minute stellate pits, and lastly, the numerous **parenchyma cells** with **leucoplasts.**

Helianthus rigidus (Perennial Sunflower).—Transverse section of young stem stained with Aniline chloride.

There is a thin-walled **epidermis** and a thickened, broad **cortex.** There is no distinct endodermis and the **pericycle** is broken up into a number of separate bundles of **sclerenchyma,** one opposite to the outer side of each vascular bundle. The latter consist, as usual, of an outer **phloem** tissue, in which the groups of small **companion cells** are easily seen ; a zone of narrow and thin-walled **cambium** cells, and a **xylem** composed chiefly of large vessels with some xylem parenchyma.

Between the bundles will be seen a narrow band of **interfascicular cambium** from which secondary vascular tissue and medullary ray parenchyma develop (see Secondary Growth, p. 207).

In the cortex may be seen a number of round openings, about the size of a single cortical cell, and surrounded by a group of noticeably smaller cells. These are sections of **oil canals** (see Internal Glands, p. 235).

Helianthus rigidus (Perennial Sunflower).—Radial longitudinal section cut through a vascular bundle stained with Aniline chloride.

The **epidermis** shows very narrow cells, about twenty times as long as broad, and the **cortex** has cells about five to eight times as long as broad. The sclerenchyma cells are thirty to forty times as long as broad and are very narrowly pointed, and they are at least partly, or in old stems wholly, lignified.

The **phloem** and **cambium** show as a band of narrow and very thin-walled cells, with occasionally a **sieve plate** visible as a thick transverse wall. The perforations of the sieve plates are difficult to see in hand sections.

The **xylem** vessels are : (1) large pitted **metaxylem** vessels with numerous small round unstained pits in their walls and with occasional transverse ribs, which mark the positions of the cross walls which originally separated the cells composing the vessel ; (2) towards the pith side of the xylem the pits become elongated ovals and pass over into a reticulate form of marking ; (3) the spiral vessels of the **protoxylem,** the spirals of which become widest in those nearest to the pith, *i.e.*, those which were first formed, as the stem xylem is **endarch.**

Aristolochia sipho (Dutchman's Pipe).—Transverse section of young stem stained in Aniline chloride.

The **cortex** is narrow, showing a mixture of large empty cells and smaller granular cells. The **epidermis** is strongly cuticularized and dark-coloured, the colour extending to the thick-walled cells of the **hypodermis.** There is a broad band of fibrous **pericycle.**

The vascular system is very large (a common feature of climbing plants) and has unusually large elements. The **protoxylem** and the large vessels, tracheids and xylem parenchyma all show well, the latter with cell contents. The **phloem** shows **sieve tubes,** some **companion cells,** and a large number of granular parenchyma cells.

Aristolochia sipho (Dutchman's Pipe).—Transverse section of old stem stained in Aniline chloride.

As is frequently the case in climbers, no continuous zone of secondary xylem is formed. The individual bundles grow radially, separated by wide **primary medullary rays.** As the bundles elongate radially they divide by the formation of **secondary medullary rays** in their midst. These, like the primary rays, are wide. **Periderm** is abundant and usually forms wings or ridges, due to unequal growth of the **phellogen** at different points of the circumference (see also *Ulmus*, p. 222).

Phaseolus multiflorus (Scarlet Runner Bean).—Transverse section of stem stained in Aniline chloride.

The **cortex** is narrow, there is no endodermis, but a **pericycle** of two to three layers of small polygonal cells with slightly thickened walls. The vascular bundles are numerous, mostly with only one radial row of vessels. Some are formed of several such rows grouped together with a common **phloem.** The **interfascicular cambium** is very clear, beginning even in the young stem.

Viscum album (Mistletoe).—Transverse section of young stem stained in Aniline chloride.

There is a broad **cortex** and a clear ring of large **vascular bundles.** In the **medullary rays** may be seen minor bundles which are branches from the larger ones.

Each large bundle shows a considerable amount of **xylem** with cells of very uniform size. The **protoxylem** elements are clearly marked, lying amid small **parenchyma** cells on the inner side of the **metaxylem,** and sometimes slightly separated from it. The **cambium** is broad and plainly evident. The **phloem** shows large **sieve tubes** and **phloem parenchyma,** but few, if any, companion cells.

Outside and inside each bundle is a well-marked strand of **sclerenchyma** cells with thick walls. There is no sign of an endodermis. **Cluster crystals** of Calcium oxalate are abundant. The **epidermis** has a remarkably thick papillose cuticle.

Hydrangea hortensis (Garden Hydrangea). Transverse section of one-year-old stem; stain in Aniline chloride.

The section shows early stages in secondary thickening and the cell elements are clear. The **pith** is large, the outer zones consisting of small thicker-walled cells. **Protoxylem** for the most part is shrunken and crumpled. The **tracheids** and **vessels** of the **metaxylem** are large, empty, and well distributed through the wood. They are approximately of equal size. Very thick-walled **xylem fibre** cells may be seen scattered here and there. **Xylem parenchyma,** with granular contents, lies mainly in radial rows; **medullary rays** are broad, their cells thickened; **phloem** and **cambium** are clear and distinct; **sieve tubes** large, appearing empty of contents. The well-marked **endodermis** consists of square cells with suberized radial walls, inside which are two to three layers of **pericycle** cells tangentially flattened. Outside the endodermis is an irregular layer of apparently cuticularized **cortex** cells, the walls of which show marked ribbing and pitting. Their function is obscure.

Lamium album (White Dead-Nettle).—Transverse section of stem stained in Aniline chloride.

The stem is square in section and the tissues are arranged somewhat peculiarly in consequence.

There are continuous zones of **xylem** and **phloem,** with a very distinct **cambium** between them. The **cortex** is very narrow, and only scattered **pericycle fibres** occur outside the phloem. There is an extra development of xylem at each corner of the section, and in the middle of each flat side and at each corner there is a large mass of thickened **collenchyma** in the cortex. Both these tissues may be regarded as extra mechanical support for the stem. The pith breaks down centrally, leaving a large hollow space.

Vicia faba (Broad Bean).—Specimen cleared to show course of vascular bundles. Cut short lengths, including at least one node in each, from strong stems of Broad Bean. Place these in 75 per cent. Lactic acid and keep them in a warm place for a week. Transfer to 70 per cent. Glycerine and leave for two or three days. This is usually enough to clear the tissues effectively, but if not, wash out the glycerine with water, which should be changed several times for twenty-four hours, and transfer to Eau de Javelle until clear. Gentle heating will complete the last stage in a few minutes. Specimens may be stained before clearing, by immersion in Basic Magenta which has been decolorized with strong Ammonia. This produces a purple colour in lignified tissues.

Place the cleared stems in a shallow dish and examine with a hand lens in a strong light.

It will be seen that the large vascular bundles at the edges of the square stem are those which form the leaf traces. Each petiole arises from one of the edges. The bundle at this edge bends outwards and becomes the lowermost petiole bundle. The bundles from the edges to right and left of the leaf base bend over sharply at the node and curve round to enter the petiole at the two upper corners. Strong branches from the first edge bundle (below the leaf) diverge and join these two latter bundles, making thus two loops, one on each side of the petiole base (see Fig. 45) from which arise the other petiole bundles. The stipules are supplied by delicate branches from the further portions of each loop.

The bundles of the main ring supply a cluster of branches to the axillary bud and then pass upwards unchanged into the next internode, except that three of them bend outwards and replace the three missing edge bundles.

The vascular anatomy of plant organs examined whole in this way reveals many peculiarities very difficult to make out in sections. The practice should be extended to other specimens, and many interesting observations may be made both in Angiosperms and in Vascular Cryptogams.

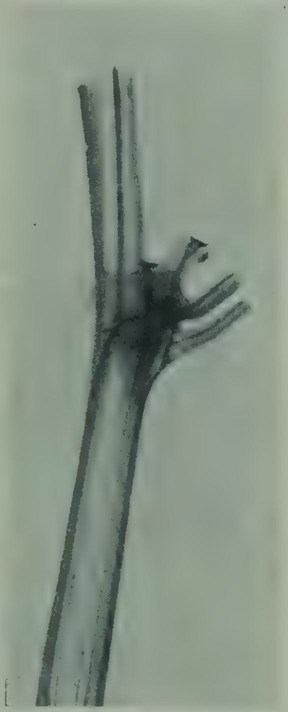

FIG. 45.—*Vicia faba.* Course of vascular bundles at the node.

2. MONOCOTYLEDONS

Convallaria majalis (Lily of the Valley).—Transverse section of rhizome stained in Aniline chloride.

As in most Monocotyledons the aerial stems of *Convallaria* are only temporary flowering shoots. We therefore turn to the underground stem (rhizome) for the true stem structure.

There is a broad **cortex** bounded on the inside by a highly thickened **endodermis** and a continuous ring of **pericycle** fibres surrounding the stele. In the stele are numerous, scattered, closed bundles of concentric structure. The **phloem** is central and surrounded by a ring of **xylem** vessels. In those nearest to the pericycle, however, the xylem ring is often incomplete. The **phloem** is of very regular structure, with **sieve tubes** and obvious **companion cells,** while the **xylem** ring consists of uniform elements with no obvious protoxylem and no xylem parenchyma.

Ruscus aculeatus (Butcher's Broom).—Transverse section of the stem stained in Aniline chloride.

The **stele** is marked by a very broad **pericycle** of heavily lignified sclerenchyma, outside of which is a fairly broad **cortex** and an **epidermis** with a thick **cuticle.** No definite endodermis is present.

The **vascular bundles** are scattered over the whole transverse area of the stele, diminishing in size, as is usual in Monocotyledons, towards the outside. Each bundle is reinforced by two bundles of **sclerenchyma,** one on each side. It is therefore a **fibro-vascular bundle.** The vascular tissue consists of a circular patch of **phloem** with small regular cells consisting of **sieve tubes** and **companion cells,** and a V-shaped mass of **xylem,** which in the larger bundles is split into two arms, or even into two portions, by a wedge of **fibres.**

The ground tissue of the stele consists of round, living cells with lignified and pitted walls and many **intercellular spaces.**

> ***Agropyron repens*** (Couch Grass).—Transverse section of the rhizome stained in Aniline chloride.

This species may be taken as typical of the Grasses. There is a well-marked, strongly thickened **endodermis,** within which are several layers of small thin-walled cells, possibly the **pericycle,** and two circles of **vascular bundles,** embedded for the most part in a layer of thickened cells. Each bundle is **collateral,** the **xylem** few-celled, with two large vessels; the **phloem** regular, **sieve tubes** and **companion cells** being clearly distinguishable. The **pith** is fistular, *i.e.*, the central part has broken down and disappeared, leaving a hollow.

In the **cortex** are many minute **leaf trace bundles.**

> ***Zea mais*** (Maize).—Transverse section of the stem mounted in Aniline chloride.

There is no distinguishable difference between stele and cortex, the vascular bundles occurring all over the section, almost to the periphery, which is formed of a few layers of sclerotic cells.

The **vascular bundles** towards the centre of the section are spaced out more evenly, and they are somewhat larger than those at the periphery, but the structure is identical in all. There is in each a small group of regular **phloem cells** with very thin walls, and three or four large lignified **xylem** vessels arranged in the form of a Y, of which the base represents the **protoxylem,** and the two arms are made up of the two large **metaxylem** elements. The protoxylem vessels normally lie loose in a cavity caused by the disruptive growth of the small parenchyma cells around the non-living vessels. The whole bundle is sheathed in **sclerenchyma.** Outer bundles are orientated with their phloem outwards, but the inner bundles have no fixed arrangement. All the bundles are common to stem and leaves, the central bundles becoming the median veins of the leaf blades, and the small peripheral bundles forming the marginal bundles in the leaf.

The ground tissue is composed of large thin-walled parenchyma.

3. STEM APEX

> ***Tropaeolum majus*** (The Garden Nasturtium).—Longitudinal section through the stem apex. For the detailed study of the tissues at the apex a series of microtome sections is almost essential.

The stem apex is surrounded by young **leaves** which arch over and enclose the growing point. These leaves are covered by a relatively thick epidermis which may develop hairs on its outer surface. The apex itself (Fig. 46) is divisible into a superficial layer which forms the **tunica,** and an internal tissue which is the **corpus.** The cells of the tunica are small and rectangular and are richly supplied with protoplasm. The nuclei are large, and there are no vacuoles. The tunica is distinguished by the absence of cross walls parallel to the surface. Its cells divide only anticlinally. The cells of the corpus are more hexagonal, but are also full of cytoplasm, and in each there is a large nucleus which may show stages in mitosis. Behind the immediate apex the cells of the corpus enlarge and become vacuolated, dividing and growing to form centrally, the **pith,** while towards the margins **procambial strands** become differentiated by progressive elongation of narrow cells which divide periclinally.

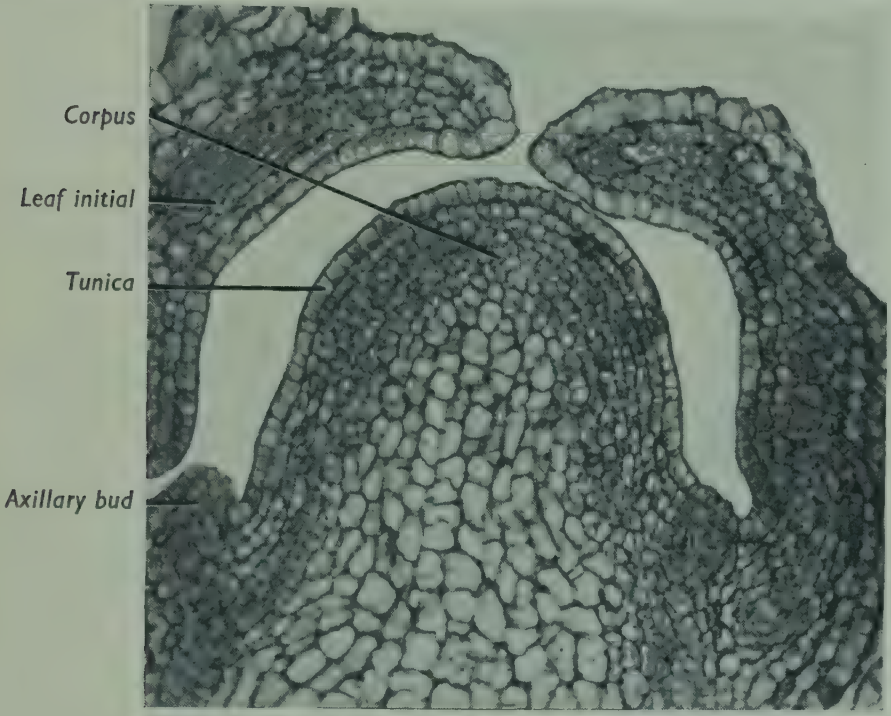

FIG. 46.—*Tropaeolum majus.* Longitudinal section of stem apex.

Just above the insertion of the uppermost leaves, swellings occur on the tunica, which are the **leaf primordia.** They are covered by the tunica, while in the young state the corpus develops a dome-shaped mass of cells immediately below it.

> ***Hippuris vulgaris*** (Mare's Tail).—Longitudinal section through the stem apex. A median section cut with a microtome is preferable.

The growing apex will be seen as a cone-shaped group of cells in the centre which constitute the **meristem.** These cells are more or less cubical in shape and each is filled with cytoplasm and has a large nucleus and no vacuole. The **tunica** forms the superficial tissue, and it is from this, farther back, that the **leaf primordia** are differentiated. As in *Tropaeolum* the central tissue forms the **corpus.** Farther back may be distinguished on the surface the small swellings which are the **leaf primordia,** while internally is the lacunar cortex in which large intercellular spaces develop. Between

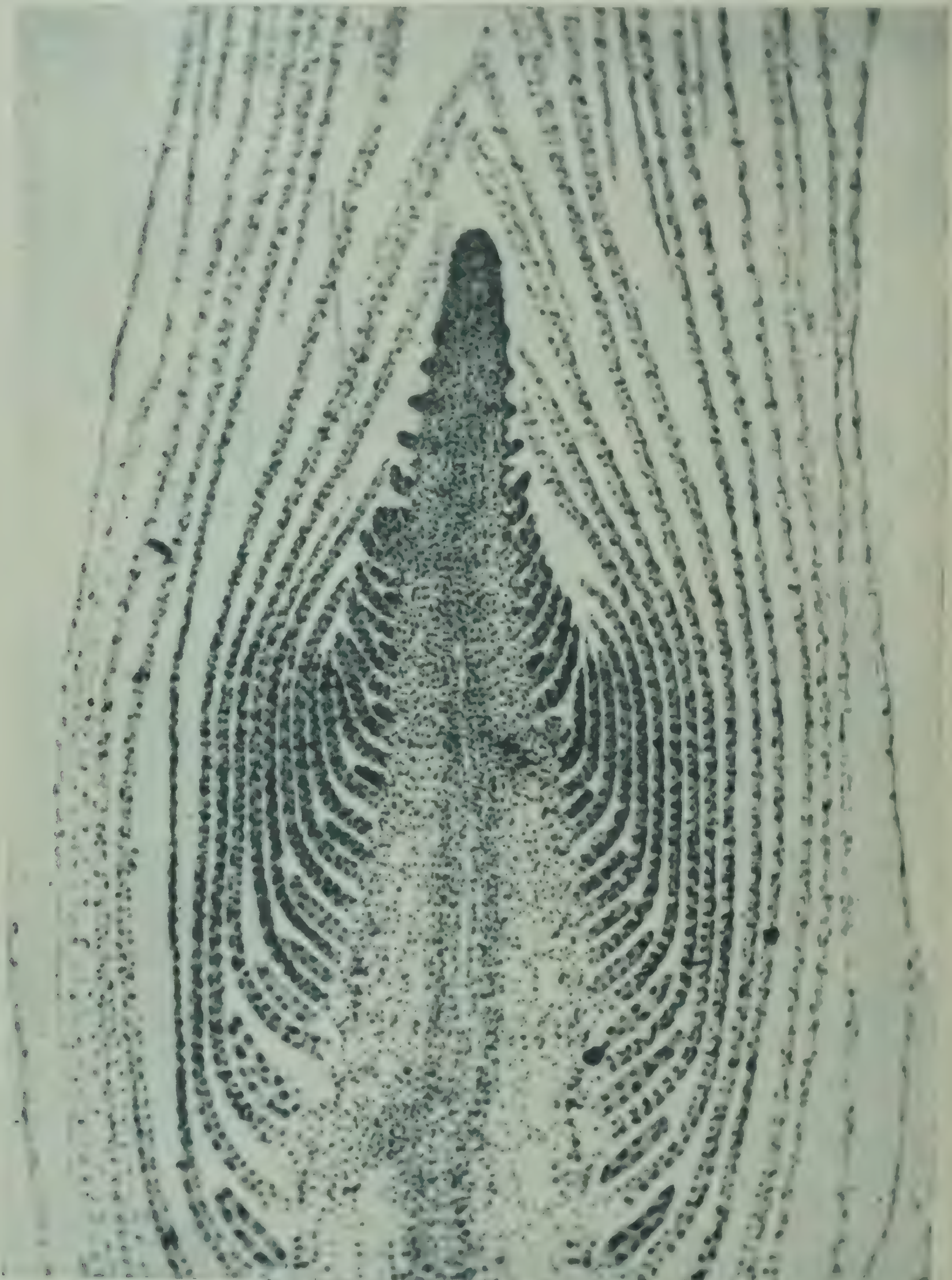

FIG. 47.—*Hippuris vulgaris.* Longitudinal section of stem apex.

this tissue and the central **medulla** or **pith** there are developed rows of elongated cells which mark the beginnings of the **procambial strands.** The vascular tissue of this water plant is poorly developed, and no lignification appears near the apex.

The **leaf initials** arise as small rounded cushions of meristem which show rapid elongation as one passes downwards from the apex itself. It may be noticed that the cells on the lower side of each initial are longer than those on the upper surface. This excess growth on one side causes the upward curling of the young leaves over the apex. In the axil of each young leaf is usually seen a knob of tissue, which is a very early stage of flower formation. Multicellular **gland hairs** may be seen developing on the leaves.

C. SECONDARY HISTOLOGY OF STEMS

1. DICOTYLEDONS

(*a*) Secondary Growth [1]

Acer pseudo-platanus (Sycamore).—Transverse section of internode of young green shoot. First stage (May to June). Stain in Aniline chloride or Chloriodide of Zinc.

Secondary thickening in trees and in plants which are shrubby or herbaceous does not always follow identical lines. Here we will trace it in a typical dicotyledonous tree.

The stem, in the primary condition, is still covered by the single-layered **epidermis,** with fairly thick cuticle. Beneath this is a wide **cortex** of smaller cells, the outer layers of which are collenchymatous. Here and there the collenchyma will be seen to form little masses of loose, rounded cells, just below the epidermis, often with a cavity among them. These are the beginnings of **lenticels.** There is no distinct endodermis. Inside the cortex comes five or six layers of small polygonal cells, mostly appearing empty. This is the **pericycle,** and these cells later become sclerenchymatous fibres.

Inside this follows the **phloem,** easily picked out under the low power by its small, dark-looking cells with dense contents. It forms a continuous band, interrupted only by the **primary medullary rays,** between the **vascular bundles.**

Next, the **cambium** is easily seen, forming a continuous band, and within that the **primary xylems,** forming isolated groups separated by the ray parenchyma. The bundles vary in size, the largest being those which will supply the leaves next above the level of the section. Each bundle has several **protoxylems.** Centrally lies the extensive **pith.**

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[1] Woody stems for sectioning should be collected in winter, since, if collected in the spring, cambial activity causes the outer tissues to break away when cut.

> ***Acer pseudo-platanus*** (Sycamore).—Transverse section of stem at the end of one year's growth (November to March); stain as above.

The **epidermis** has now been replaced by a **periderm** (bark) consisting of eight to ten layers of rectangular suberized cells of greenish-brown appearance. They arise in fairly regular radial rows from a **cork cambium** or **phellogen** at their inner margin. Outside the layer of periderm cells are seen the crushed and flattened remains of the original epidermis clinging in scraps to the surface. If the section is cut through a **lenticel** it will be seen to be open externally, and that the mass of loose cells which compose it extends right down into the phellogen.

The **cortex** is now uniformly thickened, and the **pericycle** fibres are densely lignified. There is no break in the pericycle layer, but here and there are groups of larger cells with many pits which may act as passage cells.

Within this lies the crushed **primary phloem** and a band of **secondary phloem** arising from the **cambium,** which is not so well marked in winter as during the growing season.

The band of **secondary xylem** (first annual ring) shows under the low power large, scattered **vessels,** either singly or in small groups, between which are the numerous smaller **tracheids** which, like the vessels, appear empty. At the inner margin of the wood appear the **primary xylems,** with the protoxylems protruding, wedge-like, into the **pith,** the outer layers of which are now somewhat lignified and contain starch.

Under the high power will be seen the **secondary medullary rays,** single rows of small cells filled with starch grains. Test these with Iodine run under the cover slip. These rays stretch right across the wood and can be traced farther across the cambium and phloem.

In addition to these there are small groups of **xylem parenchyma,** also filled with starch.

> ***Tilia vulgaris*** (Lime).—Transverse section of three-year-old stem; stain as above.

The **periderm** consists of very flat cells, the structure of which can only be seen properly in very thin sections. Layers of dead periderm will be seen scaling off on the outside. The **phellogen** is uncoloured; the **phelloderm** is brown. The **cortex** is very narrow and the **pericycle** is marked by large irregularly shaped cells containing crystal aggregates of Calcium oxalate. The **phloem** or " bast " is unusually broad, and is divided radially by prominent wedges of parenchyma, pointing inwards, which are the expanded outer ends of the **primary medullary rays.** Between these are the **phloems,** made up of alternate tangential layers of sclerenchyma (" bast fibres ") and of thin-walled tissue containing sieve tubes.

The **cambium,** between the phloem and wood, is evident as a band of very flat cells. The **wood** is soft, the elements rather thin-walled, and mixed with very numerous scattered parenchyma cells with protoplasmic contents. The vessels tend to run in radial rows and are formed all through the **annual rings.** There is little distinction of spring and autumn wood, only a slight increase in the amount of lignification at the outer edge of each ring. Primary, secondary and tertiary medullary rays are distinguishable, rated according to the annual ring in which they first appear. In the centre is the **medulla,** surrounded by the protoxylem elements.

Tilia vulgaris (Lime).—Longitudinal radial section through the centre ; stain as above.

In the phloem there are successive zones of **sclerenchyma** and of thin-walled tissue, containing **sieve tubes.** The **sieve plates** are usually marked by the presence, on one side, of a dense mass of coagulated tube contents, each a pyramidal mass with its broad end against the lower side of the sieve plate, the perforations in which should be noted. Note the very long and narrow **companion cells,** with a row of large pits on the wall between each of them and the adjoining sieve tube. The **cambium** shows the form of a narrow band of very narrow cells, between the wood and the phloem. **Spiral vessels** and **tracheids** make up the bulk of the wood, but note the round pits in the walls of contact between the vessels and the xylem-parenchyma, and between the vessels and the medullary rays. These are **bordered pits** (see *Pinus*, p. 165), and have only a small centre opening which may be seen as a minute spot in the centre of each pit. The vessel walls have spiral thickening bands on their inner surfaces and also have these bordered pits arranged between turns of the spirals. They are, therefore, not primarily spiral vessels.

Delicate horizontal rings may be seen at intervals in the vessels, which mark the spots where transverse partition walls have disappeared. Many wood elements in *Tilia* have no wall pattern and are smoothly lignified all over.

The **medullary rays** are seen running horizontally across the section. They are not perfectly uniform in character, but for the most part consist of oblong cells with protoplasmic contents, their long axes at right angles to the elements of the wood.

Cytoplasmic connections through the medullary ray cell walls should be looked for.

Tilia vulgaris (Lime).—Longitudinal tangential section through the phloem zone ; stain as above.

Each **primary medullary ray** has only a limited extension vertically, so the phloem bundles, which are continuous vertically, appear to form the wavy strands of a network, of which the medullary rays occupy the meshes.

Among the **phloem sclerenchyma** note the bi-convex groups of parenchyma, which represent sections through smaller (*i.e.*, non-primary) **medullary rays.** They appear as longitudinal rows of small oval cells with thick walls, sometimes in single, sometimes in double or treble series. Also note the wide channels which are the **sieve tubes.** The **sieve plates** are very oblique and are compound, *i.e.*, they consist of several sieve areas in a row, separated by bars of unperforated wall. This can only be seen adequately in a stained section. Try Soluble Blue (Aniline Blue).

If the section cuts the **cambium** it will be seen as a band of obtusely pointed, very elongated cells, with highly pitted walls.

In the wood, note the presence of **spiral vessels.** *Tilia* is peculiar in having these in the secondary wood (but see under Radial Section above).

(*b*) Structure of Mature Wood

Acer pseudo-platanus (Sycamore).—Transverse section of woody stem several years old; stain with Aniline chloride.

The **annual rings** are broad, as the tree is a rapid grower, and they are very uniform in structure. The **vessels** are in small groups of two to three together, and these are distributed evenly through the whole year's growth. The **autumn wood** formed at the outer edge of each annual ring is limited to a few layers of compressed, very thick-walled tracheids, but there is also a gradual increase of thickness in the tracheid wall observable between the beginning and end of each ring. The thin walls which are seen dividing certain vessels from one another are the oblique terminal walls, cut in the portion where the tapering ends of two vessels overlap.

The **tracheids** are very uniform in diameter, but there are a few **sclerenchyma fibres** scattered among them, of smaller diameter than the **tracheids.** The **medullary rays** are very straight and uniform, running uninterruptedly from ring to ring across the whole wood. They are mostly two cells thick, and the cells show many minute pits under the high power.

Acer pseudo-platanus (Sycamore).—Longitudinal radial section; stain as above.

This section runs alongside the **medullary rays,** which are thus seen longitudinally, crossing the vertically elongated vascular elements.

The ray cells are radially elongated, and the end walls show the numerous pits referred to above. The side walls only have pits in small groups at certain points on their walls. In fortunate sections it will be seen that the margins of the ray are formed of short cells of irregular shape and size, with very irregularly pitted walls. The other wood elements appear very much as before.

Acer pseudo-platanus (Sycamore).—Longitudinal tangential section; stain as above.

This section crosses the **medullary rays** transversely, and they are seen as short rows of small square cells, usually two cells broad but tapering to a single pointed cell at each end, *i.e.*, at the upper and lower margins of the ray. Under the high power it will be seen that the end walls of the ray cells, that is to say, those which lie in the plane of the section, are perforated by numerous minute pits, which are almost absent from the side walls. This accords with the function of the ray as a channel of radial flow of liquids. The **tracheids** of the wood show, for the most part, rather scanty pitting, consisting of a few longitudinal rows of small oval pits, while unpitted wood fibres are numerous. The **vessels** are recognizable by their large oval pits, slightly bordered, which often cover the whole longitudinal surface of the vessel. **Xylem parenchyma** consists of rectangular cells which have irregular and highly pitted walls.

Very instructive studies of the forms of the various cell-elements which make up the mature wood can be made by macerating sections cut from fresh material (see p. 11), and mounting small portions in Glycerine. The cells can be separated from one another by gentle rubbing on the cover slip.

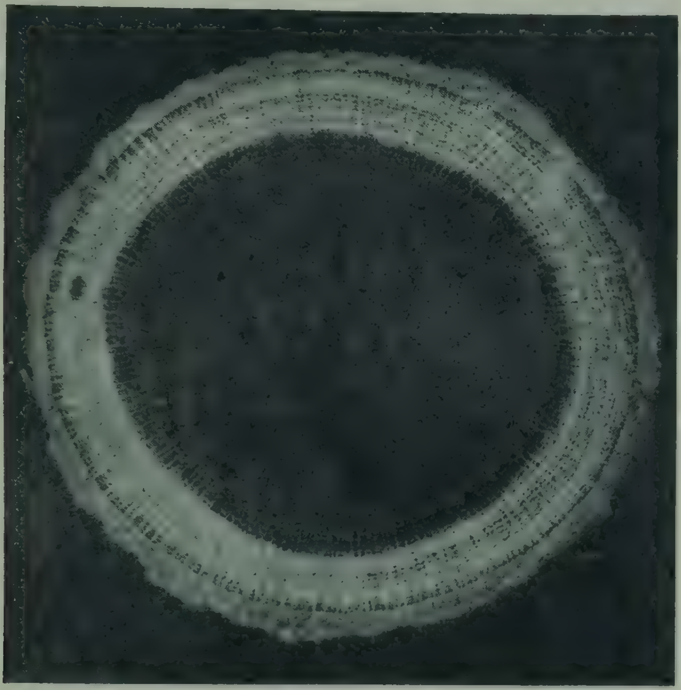

FIG. 48.—*Laburnum anagyroides.* Transverse section of wood block.

Laburnum anagyroides (Common Laburnum).—Cross-cut portion of a branch about 2 in. in diameter, with surface smoothed.

The difference in colour of the dark **duramen** (heart wood) and the light porous **alburnum** (sap wood) is very clear (Fig. 48). The heart wood contains deposits of organic waste material which fills the cell cavities and makes the wood both dense and hard. **Annual rings,** 1 to 2 mm. broad, are clearly seen in the duramen. The **vessels,** filled with light-coloured **tyloses,** are evenly distributed throughout each annual ring.

The alburnum passes over, outwards, into a brownish zone of still more porous nature, the **phloem,** while the **periderm** forms a relatively thin, hard and smooth surface layer. The outer layers of phloem with the periderm together form the **bark.**

Medullary rays, light in colour and very narrow, can be seen running from the centre to the outer limit of the phloem.

2. MONOCOTYLEDONS

Dracaena sp.—Transverse section of old stem stained in Aniline chloride.

Certain species of this genus are grown as decorative pot plants, and are obtainable from nurserymen. Most species of *Dracaena* are anatomically similar, and so are those of *Yucca* and *Aloe*.

The central part of the stem contains numerous concentric bundles with central phloems, as in *Convallaria*. This is surrounded by a zone of cells which later becomes transformed into a **cambium.** This cambium produces, on the inner side only, closely packed, but independent, vascular bundles exactly like the primary bundles (Fig. 49). A broad zone of predominantly lignified tissue is thus built up, which corresponds to the secondary wood of Dicotyledons.

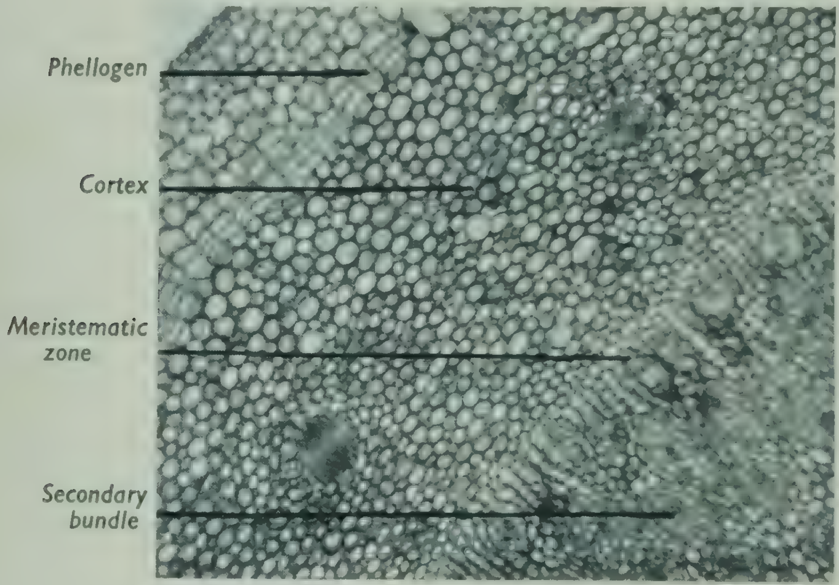

Fig. 49.—*Dracaena* sp. Transverse section of the stem to show secondary thickening.

The cells of the cortex possess very large pits with thickened margins. There is a conspicuous **phellogen,** but the **periderm** is thin and the original epidermis persists for a long time, so that even in old stems a smooth surface is retained.

Cordyline australis.—Transverse section of a stem, about 1 to 2 in. thick, stained in Aniline chloride. As the stem is large, a narrow radial wedge should be cut from it and sections cut on the transverse surface of this portion, from the periphery to as near the centre of the stem as possible.

The central part of the section shows the usual scattered monocotyledonous bundles. Each bundle contains much more **xylem** than **phloem.** The tracheids show, in longitudinal section, multiseriate bordered pits exactly like those of some Conifers.

Outside the area of the stele is a broad **cambium,** lying in the position usually occupied by the pericycle. The cambium produces complete vascular bundles, with both **xylem** and **phloem,** on its inner face, and as it expands centrifugally it leaves behind it a fairly dense array of such bundles, with some parenchyma between them. These are exactly like the primary stelar bundles, but more closely placed. This secondary growth continues indefinitely, and may sometimes show annual zones, as in a Dicotyledon. Note the **raphides** in the cortex, which are best seen in longitudinal section.

Section 2. Roots and their Modifications

A. THE EXTERNAL MORPHOLOGY OF ROOTS

1. DICOTYLEDONS

Vicia faba (Broad Bean).—The root system consists of a **main root** which grows vertically downwards and a large number of **lateral roots** which grow out almost at right angles to the main root. They are all colourless and entirely devoid of leaves or buds. There are no nodes or internodes on a true root (*cf.* rhizomes), and laterals are produced at quite irregular intervals. They arise **endogenously** from the pericycle in the root stele (see p. 216), and where they emerge a small collar of tissue may be seen, pushed outwards by the emergence of the lateral root through the cortex of the main root. Laterals may also themselves branch in the same manner, so that a complex root system is formed. If the main root is looked at endways the laterals will be seen to form five longitudinal rows, as they are formed opposite the protoxylems, and the root is pentarch (see p. 216).

With a hand lens it may be seen that the younger roots are covered with fine hairs, the **root hairs,** to which soil particles adhere tenaciously.

At the tip of each root is a **root cap,** but only an indication of this, as a yellowish region, can be seen without a microscope (see p. 36).

Ranunculus repens or ***R. bulbosus*** (Buttercup).—The root system is unusual for a dicotyledon in that the main root does not persist for long, and after its disappearance the root system consists of a number of roots of equal size which spring adventitiously from the tissues at the base of the stem. These form a bunch of parallel roots, which are very sparsely branched and bear few root hairs. They resemble in these features the roots of the monocotyledonous Onion (see p. 214).

Taraxacum officinale (Dandelion).—There is a contracted stem, with a tuft of radical leaves, from which leads downwards a single main root, a $\frac{1}{4}$ to $\frac{1}{2}$ in. thick and up to 2 ft. long. The only other roots are fibrous branches from this **tap root,** which spread laterally for some inches. If there are zones of varying moisture in the soil traversed by a tap root the drier zones will be marked by more abundant side roots and vice versa. In very rich soil the tap root may fork repeatedly, a phenomenon also shown, as gardeners know, by Carrots and Parsnips.

2. MONOCOTYLEDONS

Zea mais (Maize).—In Monocotyledons the root system is entirely adventitious. The seedling roots quickly abort, in some cases (*e.g.*, Wheat) even before germination, and are replaced by roots which grow from the base of the stem.

In the Maize, as in other grasses, these form a group of more or less equal roots, with many fine laterals which make a **fibrous system.** There is no main root.

Maize is a heavy plant, especially in fruit, and it produces many adventitious roots from the nodes just above the soil, which grow obliquely downwards and lend additional support as props.

Allium cepa (Onion).—The roots (adventitious) arise at the base of the bulb and form a long bundle of equal-sized roots. The peculiarity of these roots is that they are almost, or entirely, unbranched, growing vertically deep into the soil as is the case with most plants that are, as gardeners say, " gross feeders."

Lemna minor (Duckweed).—The minute " fronds " of this plant are of uncertain morphological character, partaking of the nature of both leaf and stem. Each bears a single adventitious root on its lower surface. These small roots hang vertically in the water. They are devoid of root hairs, a characteristic of most aquatics, but each has a relatively large and prominent **root cap,** which can be seen even with the naked eye. It is several millimetres long and fits quite loosely over the root apex.

3. MODIFIED ROOTS

Dahlia variabilis (Garden Dahlia).—The main roots are modified into large **tubers,** often weighing ½ lb., and containing **inulin** (see p. 42) as a reserve food material. These are true roots and, unlike Potato tubers, bear no buds or leaf scales.

They are supplemented by normal roots given off from the main stem, or as branches from the tubers themselves.

Hedera helix (Ivy).—This climbs by means of small **adventitious roots** which are produced in great numbers, on the sides of shoots nearest to the supporting wall or tree. They are formed only on the internodes and in a single row or in two parallel rows. In structure they agree with true roots, even bearing root hairs, and they arise endogenously from tissues of the stem stele. They apply themselves closely, by negative phototropism, to the irregularities of the supporting surface, and their attachment is aided by the breakdown of some of their outer tissues into mucilage.

B. HISTOLOGY OF ROOTS

1. DICOTYLEDONS

(*a*) Primary State

Ranunculus repens or other species (Buttercup).—Transverse section of the young root mounted in Aniline chloride or double stained and mounted in Balsam. *This is an easy object on which to practise staining.*

The greater part of the section consists of the **cortex,** formed of **parenchyma cells** with conspicuous **intercellular spaces,** and containing numerous, shining, white **starch grains.** At the circumference is the **rhizodermis** or **exodermis,** which is left as the surface layer when the piliferous layer dies. Remains of the cells of this latter layer will be seen adhering to the surface. The cells of the rhizodermis show suberization on their radial walls. In the centre is the **stele,** the core of the root, cut off from the cortex by the **endodermis,** the radial walls of which are also suberized. The suberization takes the form of a ring in the middle lamella round each radial wall, the central part of which is unaltered. This allows lateral movement of fluids from cell to cell, but prevents radial movement through the walls. Within the endodermis lies the **pericycle,** a single layer of small thin-walled cells, best seen between the endodermis and the **xylem,** which forms four or five groups of conspicuous lignified cells. The smallest cells of each group are on the outer edge, and constitute the **protoxylem,** while the larger elements (vessels) form the **metaxylem.**

In the young stage the centre of the root is occupied by a group of large thin-walled cells which indicate immature vessels. In older stages these are lignified like the others. There is no pith. Between the xylem groups lies the **phloem,** each group of these thin-walled cells alternating with a group of xylem cells.

Plantago media (Plantain).—Transverse section through a mature root stained in Aniline chloride.

There is a surface layer, the **exodermis,** the outer walls of which are, at least partially, suberized. Remember that this is not a true epidermis, since it arises below the original surface layer of piliferous cells, which only last for a short while.

The cortical cells are neatly arranged in radial rows, with large intercellular spaces, but the endodermis is not easily made out unless stained differentially, as suggested under *Vicia* root (see p. 216). The **Casparian band** is on the radial walls of the endodermal cells.

There are five primary xylems, each little group with about half a dozen elements. The phloem groups are distinguished by their somewhat thicker walls and denser contents from the **conjunctive tissue** which occupies the centre of the section.

> ***Vicia faba*** (Broad Bean).—Transverse section of a root 3 mm. in thickness stained in Aniline chloride. Roots of this thickness are in a transitional stage, showing the beginning of secondary thickening.

The root is usually pentarch, with large groups of **primary xylem** cells and a small central area of **conjunctive tissue.** Alternating with the primary xylems are the masses of early **secondary xylem,** which are easily distinguished by their smaller elements, not so densely lignified as the large vessels of the primary xylem. At the outer margin of each secondary xylem will be seen an arc of narrow **cambial cells,** which may be traced sideways as far as the protoxylems. In older roots (about 5 mm. in thickness) the cambium may be seen passing round outside the protoxylems and thus forming a continuous band round the stele. In these younger roots, however, this stage has not as a rule been reached.

The **primary phloems** lie externally to the secondary phloems, but they are scanty and not easily distinguishable from the cells of the **pericycle,** except for the triangular thickenings (**trigones**) at the corners of the cells.

The **endodermis** has a very pronounced **Casparian band** of suberin on its radial walls. If a section is laid in a watch-glass in dilute Glycerine, which is slightly coloured with Safranin and left for some hours, the Casparian band will be stained selectively, showing up red, while the rest of the root is still unstained.

> ***Ranunculus repens*** (Buttercup).—Longitudinal section of a young root stained in Aniline chloride.

The appearance of the tissues in longitudinal section will depend on the radius in which the plane of the section lies and also on whether the section is tangential or radial to the small, central stele.

The **exodermis** consists of flattened and somewhat collapsed cells with brownish, suberized walls. The cells of the **cortex** are large and mostly filled with **starch grains.** The **endodermis** and **pericycle** consist of narrow, elongated cells ; the former having numerous pits. The **phloem** has long, narrow cells in which the oblique sieve plates are conspicuous. The **protoxylem** elements, if present in the section, may be recognized by their spiral thickening, while the **metaxylem** is chiefly composed of vessels of various diameters, bearing numerous oval-bordered pits with slit-like openings.

> ***Vicia faba*** (Broad Bean).—Transverse section through the root to show the origin of lateral roots. Such a section is best made from the main root of a seedling, and the branches should be as young as possible.

The bean root is usually pentarch and root-branches arise opposite the primary xylems. The initial development is a meristematic cushion formed by tangential cell-division in the pericycle. This rapidly elongates in a radial direction, pushing through the cortex by separating or dissolving the parenchyma, and finally erupting through the root surface. The root-cap may already be seen before the surface is reached, and the young vascular strands shown as dark lines near the median axis of the rootlet. The xylem of the lateral branch is attached to the secondary wood on each side of that primary xylem of the main root which lies opposite the base of the lateral. The primary xylem itself takes no direct part in the branch formation. Between the primary xylem groups may be seen groups of phloem fibres with thick laminated walls.

Sinapis alba (White Mustard) or ***Lepidium sativum*** (Cress).—Seeds germinated for root-hairs.

To get clean **root-hairs** for examination the seeds should not be sown in soil or sawdust. Coconut fibre is better, but best of all is to sow the seeds on wet blotting-paper in a glass dish or covered saucer. Another very good method is to sow the seeds on pieces of clean, broken flower-pot, placed in a shallow layer of water in a saucer. The rootlets then develop in the air and show a fine growth of root-hairs.

Cut off a piece of root covered with hairs, including the apex, and mount in water. Examine with the low power and note that the development of the hairs starts a few millimetres back from the tip, each hair coming from a single cell of the **piliferous layer.** Growth is rapid, and the hair reaches its full length (about 15 mm.), at about 10 mm. back from the point where it began to grow. It persists only for a short time and falls off, taking with it the cell from which it grew, at a distance of only a few centimetres from the root apex.

Under the high power the root-hairs will be seen to have a thin cellulose wall, with a rounded, or sometimes a distorted tip, and to contain highly vacuolated cytoplasm. Usually there is only a thin protoplasmic utricle and one large central vacuole, except at the tip. The nucleus may be seen vaguely in the living state, or more clearly after adding Iodine, usually near the tip. Cyclosis may sometimes be seen, especially if the cell is mounted in a very weak solution of Sodium bicarbonate. As the hair gets older the cytoplasm gets more solid, and eventually appears to be gelatinous.

(*b*) Secondary State

Cucurbita pepo (Vegetable Marrow).—Transverse section stained in Aniline chloride.

The **secondary wood** forms three or four large wedges with broad **primary medullary rays** between. At the centre, where the points of the wedges meet, look for the **primary xylem** strands which are equal in number to the secondary xylems but alternate with them, *i.e.*, they lie opposite the inner end of each of the large medullary rays. The primary xylems consist of very small elements. They can best be seen under a high power, and a good section is necessary. There is often a single central vessel from which the primary xylems radiate, their **protoxylems** directed outwards.

In the secondary xylems, notice the very large **vessels,** each surrounded by lignified **tracheids** and separated by masses of unlignified parenchyma cells. In old roots **tyloses** are common in the vessels. There is a broad **cambium,** five or six cells thick, and massive **phloems,** with large sieve tubes in which **sieve plates** may often be seen, the pores being clearly visible. The outer phloem contains many yellow-coloured tannin cells. The cambium does not extend across the medullary rays, which grow by the radial extension of their cells. The **cortex** is slightly collenchymatous. There is no true periderm, the external layer of cells, the **rhizodermis,** being slightly cuticularized.

Cucurbita pepo (Vegetable Marrow).—Longitudinal section stained in Aniline chloride.

The longitudinal section of the Marrow root shows very instructive details of vascular structure. The large vessels have a " concertina " or " bellows " structure, which prevents their lateral collapse, each constriction of the wall being strengthened by a heavy ring of lignin. Between the constrictions the walls are thickly covered with bordered pits, each with a slit-like opening.

Each vessel is surrounded by a sheath of very small, highly lignified cells with abundant pitting on their walls. They presumably act as reservoirs for the vessels. Beyond these come the lignified **tracheids** with narrow bordered pits and the unlignified **xylem parenchyma.** Cross sections of sieve plates are easily seen in the sieve tubes nearest the cambium. Those farther off, *i.e.*, older, are out of action. They are usually collapsed, and their sieve plates are thickly covered with **callose.**

Ulmus procera (Hedgerow Elm).—Transverse section of a root of about 2 mm. in thickness stained with Aniline chloride.

At the centre is a small mass of parenchyma, roughly triangular in section, with three **primary xylems,** one in each corner of the triangle. There are also a few scattered vessels, associated more or less loosely with the primary xylems.

NOTE.—The above arrangement with *three* primary xylems (*triarch*) is typical, but roots with four (*tetrarch*) or with two (*diarch*) may also be found.

Opposite each primary xylem is a broad **primary medullary ray,** lignified in its inner portion, but spreading fanwise and losing its lignification as it passes outwards through the phloem.

The **secondary xylem** has numerous large vessels and abundant xylem parenchyma. The **cambium** is clearly visible as a band of narrow thin-walled cells surrounding the outside of the xylem masses and bridging across the medullary rays so as to form a complete circle.

The large **phloem** masses contain numerous sieve tubes with dense contents. There are also numerous small nests of sclerenchyma fibres and rhomboidal crystals of Calcium oxalate mixed with thin-walled phloem elements.

The **periderm** lies immediately outside the phloem, *i.e.*, it has been formed in the pericycle, so that all cortical tissue has been lost. The narrow cells of the **phellogen** and the square, yellowish-walled cells in radial rows, which form the cork, are well shown.

Ulmus procera (Hedgerow Elm).—Transverse section of an older root about 5 mm. in diameter.

The chief differences from the younger stage are : much more extensive **secondary xylem** with numerous, narrow, **secondary medullary rays** and larger **phloem** masses, in which the large sieve tubes are arranged in roughly tangential rows, between which are sclerenchymatous groups and flattened, crushed **phloem parenchyma.** The medullary rays will be seen to run right out to the periderm, the cells of which are narrow-rectangular in shape, like the phellogen cells.

Very good **tyloses** may be seen in many of the vessels of the secondary xylem, showing clearly, in many cases, their origin from neighbouring xylem parenchyma cells.

The roots of the Virginia Creeper (*Ampelopsis veitchii*) also show very good tyloses.

2. MONOCOTYLEDONS

Iris germanica (Purple Flag Iris).—Transverse section of young root stained in Aniline chloride.

The external layer or **rhizodermis** consists of three rows of rhomboidal cells with suberized walls and no cell contents (*cf.* velamen). Within this comes the broad **cortex,** of rounded closely-fitting cells, containing rounded **starch grains.**

Surrounding the **stele** is the **endodermis,** a single layer of cells, which in young roots appear oblong with slight thickening on their side walls, but in old roots have a well-marked thickening both on side and inner walls. Opposite the protoxylems occur unthickened **passage cells,** but in old roots these too may be thickened like the rest.

The **pericycle** is a single layer of thin-walled cells, separating the endodermis from the **protoxylem** groups, of which there are about fifteen. Alternating with the protoxylems are the **phloem** groups which, in young roots, are distinguished only by their rather larger cells (sieve tubes) from the **conjunctive tissue** which surrounds them and which also forms the **medulla** in the centre of the stele. In older roots, however, the conjunctive tissue becomes lignified throughout the stele.

Large **metaxylem vessels** will be seen either forming or formed on the inner side of each protoxylem.

Zea mais (Maize).—Transverse section of a prop root about 5 mm. in diameter ; stain with Aniline chloride.

The stele is relatively large and the **endodermis** is clearly seen, composed of cells with the inner half of each cell thickened, as in *Iris*. Unlike the latter, however, there do not appear to be any unthickened passage cells in this endodermis, which must, therefore, constitute an almost impermeable barrier between stele and cortex. The cells of the **cortex** become gradually smaller and thicker-walled as one proceeds outwards, so that the mechanically strongest tissue is nearest the circumference, an arrangement which gives maximum resistance to bending strains. Inside the stele are fifty or more **protoxylems,** accompanied by a small number of very large **metaxylem** vessels, arranged in a narrow zone round the large central **medulla** or conjunctive tissue. These vessels have pitted walls which are only lightly lignified. The **phloem** can only be picked out between the protoxylems by the protoplasmic contents of its cells, which otherwise resemble very closely the neighbouring cells of the conjunctive tissue.

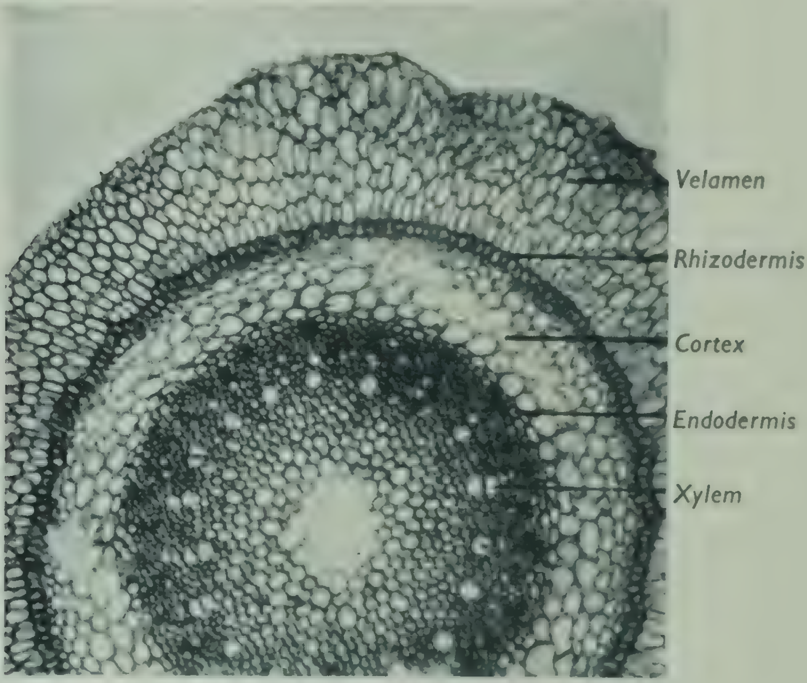

FIG. 50.—*Vanda teres.* Transverse section of an aerial root.

Epidendrum sp., Vanda sp., or ***Grammatophyllum sp.***—Aerial root of epiphytic Orchids showing velamen.

Externally, these aerial roots have a dead white appearance, due to an outer covering of empty cells which is called the **velamen.** The cells are not only empty, but in most cases the walls are also porose, so that the tissue is like a sponge. Functionally it takes the place of the root-hairs as an absorptive agency when water is available, while in dry weather it acts as a protective layer over the living tissues within.

A transverse section, cut fairly thickly and stained with Aniline chloride, shows a **velamen,** consisting of a layer five or six cells deep, on the outside (Fig. 50). The cell walls are thin and unstained, but they are marked with a network of fine supporting **ribs,** and show large open **pores** connecting the cells. The outer surface is slightly cuticularized. Inside the velamen is the **rhizodermis,** a single layer of cells with suberized walls, slightly stained. Here and there in this layer are unsuberized cells, the **passage cells,** in which invading parasitic organisms may sometimes be seen. Within this, again, comes, in order, the broad **cortex,** the **endodermis** with unthickened passage cells opposite each protoxylem, the **pericycle,** which is lignified except immediately under the endodermal passage cells, and twenty to fifty xylem and phloem groups, the latter surrounded by **sclerenchyma.** In the centre is the **conjunctive tissue.**

Section 3. Periderm Development

Pyrus communis (Pear).—Transverse section of stem of current year's growth stained in Aniline chloride to show epidermal periderm.

The **phellogen** originates in the epidermis, so that none of the cortex is affected by periderm formation. In a section of a twig several months old the circumference consists of two or three layers of radially superimposed cells, with dark orange-coloured contents consisting chiefly of tannin. Beneath these lies a single layer of very narrow thin-walled cells, the **phellogen.** Each layer of periderm cells has thin lateral walls and thick tangential walls which are suberized. See page 46 for tests for suberin which may be applied here.

Fagus sylvatica (Beech).—Transverse section of stem of current year's growth stained in Aniline chloride for hypodermal periderm.

The periderm consists of a few layers of yellowish cells of irregular section, outside of which may be seen the original epidermis, the cells of which are more oval than those of the cork cells. This indicates that the phellogen arose immediately below the epidermis, *i.e.*, in the outermost layer of the cortex. The narrow flattened cells of the **phellogen** are seen at the inner margin of the periderm, and inside the phellogen are a few layers of **secondary cortex,** with thickened walls, which have been cut off centripetally from the phellogen.

The primary cortex shows irregularly distributed tannin cells with dark contents.

Acer pseudo-platanus (Sycamore).—Transverse section of the stem towards the end of the first year's growth to show cortical periderm.

See description above, under Dicotyledon stem, secondary thickening, page 208.

Ribes rubrum (Red Currant).—Transverse section of stem of one year's growth stained in Aniline chloride to show deep-seated pericyclic periderm.

The periderm appears as a band of yellowish suberized cells which are flattened tangentially so as to form a complete band of suberin. Outside this some remains of the exfoliated cortex and epidermis can be seen. The **phellogen** is made up of several layers of very flat cells, within which lies a single layer of large oval cells in contact with the phloem.

There is thus no cortex internal to the periderm, which may be called "pericyclic," as it corresponds in position to the pericycle.

Berberis vulgaris (Barberry).—Transverse section of the stem of one year's growth stained in Aniline chloride to show pericyclic periderm.

The **phellogen** arises immediately interior to the very broad zone of pericyclic **fibre cells,** which are larger than most sclerenchyma, and are conspicuous from their lignified walls. Outside the fibre zone there is a very narrow band of cortex and the epidermis. Inside the fibre zone is seen a layer of thin-walled, suberized, periderm cells, mostly distorted in form. Within this lies the phellogen, and within this again a zone of oval cells with dense contents, which represents **secondary cortex** (see *Fagus*).

Sambucus nigra (Elder).—Transverse section of the stem of second year's growth taken through a lenticel.

The **phellogen** is formed in the layer of cells immediately below the epidermis, and the latter persists for some time after cork formation begins, giving the stems a smooth surface. The phellogen cells are like cells of the wood cambium, flattened radially, very narrow and thin-walled. From each cell of the phellogen there is cut off a succession of **periderm** cells, which have therefore a regular arrangement in radial rows. The periderm cells are themselves thin-walled and yellowish in colour owing to suberization. Outermost of all is seen the dead but persistent remains of the epidermis.

Where a **lenticel** is to be formed the phellogen is increased in thickness into a bed of meristematic cells as much as a dozen layers deep. From this is produced externally a mass of small, rounded, suberized cells in such quantity that they burst open the overlying tissues and form a raised cushion of a dark colour, easily seen, on the surface of the stem. The rounded cells do not fit closely together, hence the mass is porous and permits the passage of air to the inner tissues, whereas the true cork layer is quite impermeable. These specialized lenticel cells are called the **complementary tissue.**

Lenticel tissue is usually of a different colour from the rest of the bark, sometimes lighter, sometimes, as in this case, darker.

In older stems it may be observed that there are a few layers of cells arranged in radial rows, internal to the phellogen as well as external. This is **secondary cortex,** formed inwards from the phellogen and known as **phelloderm.**

Cells in the pith with reddish-brown solid-looking contents are **tannin cells.**

Ulmus hollandica (Dutch Elm).—Transverse section of a branch with cork wings. Sucker shoots from the base of the tree usually have these wing-like outgrowths of cork. *Acer campestre* also has similar cork wings on the young shoots.

The **phellogen** grows with very unequal rapidity at different points of the stem circumference. The result is that cork is formed chiefly as a number of wings or ridges composed of regular radial rows of suberized cells. At the inner edge of each wing is seen an active **phellogen** several cells in thickness. Between the ridges the periderm is comparatively thin. The suberization in the cork wings is relatively slight and the tissue is consequently known as **phelloid.**

NOTE.—In the secondary wood of *Ulmus* the vessels occur in small groups, and round each group will be noticed a well-marked ring of **xylem parenchyma,** which is easily seen.

Aucuba japonica or ***Prunus lauro-cerasus*** (Cherry Laurel).—Transverse section of leaf for the development of wound cork. Examine the leaves on a bush for injuries, particularly holes eaten out by insects, and cut a section across the margin of the injured region.

The injured surface is covered with shrivelled remains of dead cells, behind which there is built up a tissue of cork cells, two or three layers thick, from a delicate **phellogen** formed from the mesophyll cells.

Section 4. Leaves and their Modifications

A. EXTERNAL MORPHOLOGY OF LEAVES

1. DICOTYLEDONS

(*a*) Simple Leaves

Syringa vulgaris (Lilac).—The leaves are **ovate, entire** and **petiolate,** that is, each possesses a distinct leaf stalk. They are set in **opposite pairs.**

Veronica sp.—Any shrubby species, or almost any Labiate. Leaves set in **opposite pairs,** each pair being at right angles to the pair above and the pair below it.

Asperula odorata (Woodruff).—The leaves are **simple** and **oblanceolate,** *i.e.*, with the broadest part towards the distal end. Each leaf tapers abruptly to a short point (**mucronate**). The leaves are arranged in **whorls.**

Fagus sylvatica (Beech).—The leaves are **simple, ovate, lanceolate** and **serrate,** with **pinnate venation.** Each leaf has two narrow, delicate **stipules,** at the base of the **petiole,** which are shed before the leaf reaches maturity.

Quercus robur (Oak).—**Pinnatifid** or **sinuate** leaf, broadest towards the apex, narrowing down to the base ; margin with irregularly rounded lobes.

Taraxacum officinale (Dandelion).—**Pinnatifid, runcinate,** apex broad, obtuse, the margin very irregularly toothed, as if roughly torn.

Capsella bursa-pastoris (Shepherd's Purse).—The basal (radical) leaves are mostly **pinnatifid,** but are very variable in form. The stem leaves have part of the lamina prolonged downwards and embracing the stem. This is called **amplexicaul.**

Tropaeolum majus (The Garden Nasturtium).—This has a **peltate** leaf, with the stalk attached to the under surface, not to the edge of the lamina.

Hedera helix (Ivy).—This is a good example of a **palmate** leaf, in which the lamina is partly divided into **spreading lobes.** The leaves on the flowering shoots are, however, entire.

Aconitum napellus (Monkshood).—This is also a **palmatifid** type, though the divisions are carried down almost to the base of the lamina. Only if the divisions separate the segments completely to their bases is the leaf considered compound.

(*b*) Compound Leaves.

Fraxinus excelsior (Ash), ***Sambucus nigra*** (Elder) or ***Rosa sp.*** (Rose). — All these provide examples of **compound leaves,** in which the individual leaflets are arranged **pinnately,** *i.e.*, in opposite pairs. There is also an unpaired terminal leaflet, so that the leaf would be termed **imparipinnate.**

Ranunculus repens (Buttercup).—Each leaf comprises an opposite pair of short-stalked lateral leaflets and a longer-stalked terminal leaflet. Each leaflet is **palmately** divided into three lobes by deep divisions, and each lobe is also toothed. A compound leaf with three leaflets is called **ternate.**

Trifolium pratense (Clover) and *Potentilla reptans* (Cinquefoil) are alternative examples of similar type.

Aesculus hippocastanum (Horse Chestnut).—A **compound, digitate** leaf, the segments arranged like the fingers of a hand, and each joined separately to the apex of the petiole. The segments are usually **mucronate,** that is, they taper abruptly into a short, narrow point.

Lupinus polyphyllus (Herbaceous Lupin) is similar.

2. MONOCOTYLEDONS

Avena sativa (Oat).—The leaves of grasses usually show two distinct portions, the **sheath,** which originates at a **node**, usually marked by a swelling on the stem, and the **limb** or **blade** of the leaf. The sheath is wrapped closely round the stem, sometimes for a height of several inches, while the limb stands out at an angle. Between the two is a joint, at which there is a short, erect, membranous structure, the **ligule.**

Most grasses illustrate the same type of morphology as the above, but there is considerable variation in the development of the ligule, which is one of the most useful vegetative characters for the identification of grasses.

Convallaria majalis (Lily of the Valley).—The leaves arise on short lateral branches of the rhizome, the lowest leaves of which form sheathing scales or **kataphylls.** Usually only one or two leaves of the branch develop fully, their lower portions being rolled into tubular form, enclosing the undeveloped upper leaves and sometimes a young terminal inflorescence. The leaf expands gradually upwards into a **lanceolate lamina** with numerous **parallel veins,** some **primary,** which run from base to apex, others, smaller and **secondary,** which disappear as base or apex is approached. There are a relatively small number of horizontal **cross-connections** between the longitudinal veins.

3. OTHER TYPES OF FOLIAR ORGANS

Ribes sanguineum (Flowering Currant).—In spring the opening buds show a very interesting series of transitional stages, connecting the typical bud scales with the typical leaves, thus illustrating the leaf nature of the scales (see *Aesculus*, p. 197). The outermost (lowest) scales are very small, but the inner ones are much longer and thinner and more membranous. As one goes farther inwards these scales show the development of a true but minute leaf lamina at their tips, and the farther into the bud one goes the larger the lamina portion becomes relative to the scale portion. Buds in different stages of opening should be examined, and they will yield various forms which make an uninterrupted series between the minutest outer scales and the fully developed leaves, in which the scale portion is seen to be equivalent to the expanded base of the petiole. Such reduced leaf structures at the base of a shoot are called **kataphylls.**

Syringa vulgaris (Lilac) is another good example of the same thing, except that in this case the transitional stages show the development of a petiole by the scales, the flattened portion of the scale passing over gradually into the leaf lamina.

Rosa sp. (Rose).—The petioles have each a pair of leaf-like structures attached, one on each side at their bases. These are called **stipules** (*cf.* Pea and Bean). They develop before the leaf which, in its youngest stage, is folded between them and thus protected. When the leaf is fully developed they are no longer of importance, and in many plants they are then dropped.

Stipules are not of universal occurrence and are characteristic of certain families, while entirely absent from others.

Ribes uva-crispa (Gooseberry).—The spines are often said to be **stipules,** but the family to which the Gooseberry belongs (Saxifragaceae) is otherwise devoid of stipules, and it seems probable that they are here really outgrowths of the base or pulvinus of the petiole.

4. PHYLLOTAXIS

Phyllotactic arrangements of leaves on a shoot may easily be illustrated by sticking pins, representing leaf insertions, into a short length of broomstick. In this way the common types: opposite, decussate or alternate, and the simple spirals such as one-third, two-fifths and three-eights may be copied, illustrating, for example, that each type of spiral depends upon the angular divergence between successive leaves, so that in a one-third spiral each leaf is separated by $360 \div 3 = 120^\circ$ from its neighbour.

B. HISTOLOGY OF LEAVES

1. DICOTYLEDONS

Helianthus annuus (Sunflower).—Transverse section of the leaf blade stained in Aniline chloride.

There is an upper and lower **epidermis** between which lie the tissues comprised under the name **mesophyll.** Both epidermes have a thin layer of external **cuticle,** but have no chloroplasts. In the lower epidermis there are numerous **stomata.** These are marked by the presence of a pair of small squarish cells with thick walls, the **guard cells,** much smaller than the other epidermal cells. In favourable sections the aperture between them, the **stomatal pore,** is well seen, but many sections pass tangentially through the stomata, missing the pore, so that their position can only be inferred from the presence of the small guard cells. Stomata also occur in the upper epidermis, though fewer in number.

The mesophyll consists of two distinct portions. The upper, the **palisade layer,** has narrow elongated cells, with numerous **chloroplasts** arranged on the longitudinal walls. There are usually two layers of such cells. The lower portion, the **spongy** or **lacunar mesophyll,** is made up of cells of irregular shape, touching one another only at a few points and having, therefore, an extensive system of intercellular spaces, like a sponge, which is in communication with the outside air through the stomata, and which also extends upwards between the palisade cells.

In the median plane of the section are transverse or oblique sections of the leaf veins or **vascular bundles,** with small groups of **xylem** (uppermost) and of **phloem** (lowermost) cells. Above and below each vascular bundle a patch of colourless cells reaches to the epidermis, so that the veins appear colourless when viewed against the light in the fresh leaf. This colourless tissue is supposed to act as a passage tissue between the mesophyll cells and the vascular bundle, and is spoken of as the "glucose sheath" of the bundle.

Here and there short hairs, formed of a single row of cells, are seen on both upper and lower epidermes.

Ligustrum vulgare (Privet).—Transverse section of the leaf stained in Aniline chloride.

Upper and lower **epidermes** are single-layered and are covered with **cuticle,** which is noticeably thicker on the upper surface, *i.e.*, that which is exposed to the sun. The **palisade cells,** about eight times as long as broad, are mostly in a single layer, with chloroplasts lining the long walls. The **spongy mesophyll** is broad and well developed, as in most evergreen or semi-evergreen leaves.

The amount of intercellular space varies a good deal with the degree of exposure of the leaf, but usually is less abundant than in the Sunflower. **Stomata** are found on the lower surface only, with small **guard cells,** highly thickened on their inner and outer surfaces.

There are also small glands on the lower surface, each consisting of a cushion of four cells, slightly sunken, so that the top of the gland is flush with the outer surface of the epidermis.

Note that the cells of the spongy mesophyll which lie next to the palisade cells are broader at their upper ends, and that each makes contact with more than one palisade cell. These cells are sometimes called " collecting cells," and are supposed to act as physiological links between the spongy mesophyll and the palisade layer.

The **midrib** shows a single crescent of vascular tissue, with the xylem uppermost, *i.e.*, on the concave side.

Fuchsia macrostemma, F. riccartoni, or other hardy species.—Transverse section of the leaf stained in Aniline chloride.

The leaf is relatively thin, the upper **epidermis** formed of large cells and the lower epidermis of much smaller cells. Both epidermes are devoid of chloroplasts. There is only one layer of **palisade cells,** each slightly tapering downwards. The **spongy mesophyll** is very lacunar and is sharply marked off from the palisade. Here and there are large cells containing mucilage and bundles of **raphides,** *i.e.*, needle crystals of Calcium oxalate.

The **stomata** are small and occur on the lower surface only, the **guard cells** lying flush with the surface of the epidermis. On the lower surface are scattered **hooked hairs,** with thickened walls.

Rhododendron ponticum.—Transverse section through the midrib of the leaf stained in Aniline chloride.

Both upper and lower **epidermes** are double-layered, and there is a thick **cuticle,** especially on the upper surface. The second epidermal layer is called the **hypodermis.** The **palisade layer** has five or six layers of rather short cells, which is characteristic of evergreen leaves. Here and there are cells with **cluster crystals** of Calcium oxalate. The **spongy mesophyll** has very large intercellular spaces, contrasting markedly with the very compact palisade tissue.

The **midrib** shows a crescentic mass of vascular tissue, with a **cambium,** indicating that it undergoes secondary increase in size, while across the top of the crescent lies another vascular band with reversed orientation, the xylem pointing downwards. The two vascular masses are independent of each other, but they are surrounded by a common sheath of thickened fibre cells.

Ilex aquifolium (Holly).—Transverse section of the leaf stained in Aniline chloride.

The **epidermis** has a very thick **cuticle,** about half the thickness of the epidermis itself. Beneath this, on the upper surface of the leaf, is the **hypodermis** of thin-walled cells, probably for water storage, and a **double palisade layer** of rather short cells. The **spongy mesophyll** is relatively bulky and occupies three-quarters of the thickness of the leaf, the intercellular spaces being particularly large. The vascular bundles, especially the midrib, have strong **sclerenchymatous sheaths,** which do not, however, completely encircle them. **Cluster crystals** of Calcium oxalate occur near the midrib. **Stomata** are formed on the lower surface only, sunken in the epidermis, and with a very narrow pore.

Populus nigra or ***P. serotina*** (Poplar).—Leaf skeleton, obtained by packing dead leaves together in a closed tin until rotten and then steeping in water until the soft parts may be easily washed away.

Note the midrib and the principal branch veins, which give rise to the minor branches of the network, which in turn divide up the space between them into areolae of approximately equal size. Into each such area runs a short vein or vascular bundle, sometimes two or three, which branches and ends blindly. This network offers the possibility of many alternative routes for the supply of water if any portion is accidentally damaged. A continuous vein forms the actual leaf margin.

2. MONOCOTYLEDONS

Lilium candidum (Madonna Lily).—Transverse section of the leaf stained in Aniline chloride.

The **epidermis** is similar on both sides, with large rectangular cells and thickened **cuticle.**

There is no clear differentiation in the mesophyll, the whole tissue having a more or less lacunar character. Immediately under the epidermis the cells are closer together and have more chloroplasts than in the middle of the leaf, but there is no true palisade.

Stomata occur on both surfaces. They have exceptionally large guard cells, and the pore shows an inward and an outward expansion, with an overhanging lip on each side.

The **vascular bundles** are numerous but small, and as they run parallel to each other they are all cut transversely.

Narcissus majalis (Narcissus).—Transverse section of the leaf stained in Aniline chloride.

The section is broadest in the middle and tapers to both edges. **Stomata** occur on both surfaces and are large, so that details of the pore are easily seen. In section the **pore** looks dumb-bell shaped, with an outer expansion, arched over by the outer edges of the guard cells ; a narrow channel midway down and an inner expansion, the edges of which are turned outwards and upwards. The epidermis has a thin, ridged **cuticle** and beneath it lies the assimilating tissue, with one layer of short **palisade cells** and two layers of **chlorenchyma,** representing the spongy mesophyll.

In the middle of the section lie eight to twelve primary **vascular bundles,** of the simple collateral type, the smallest being nearest to the leaf edges. Between these are groups of very large thin-walled **water storage cells,** which break down to leave hollows filled with dilute mucilage.

Secondary bundles lie between the primaries, towards the abaxial side, while very small **tertiary bundles** are found close to the chlorenchyma, all round the leaf. All are linked to the primary bundles by connecting strands, running at approximately right angles to the main bundles.

Avena sativa (Oat).—Transverse section through the leaf stained in Aniline chloride.

This section shows the structure of a typical grass leaf. The leaf is thin, and both epidermes are similarly composed of rather large cells with thick **cuticle.** There are **stomata** on both sides of the leaf, the guard cells being small and highly thickened, with two **subsidiary cells,** one on each side of the stoma (see p. 231 for the surface view of a typical grass stoma). Between the vascular bundles the cells of the upper (adaxial) epidermis are enlarged into **water storage cells.** These cells collapse readily under conditions of drought, and hence cause an inrolling of the leaf which tends to protect the adaxial leaf surface.

The mesophyll is dense, with a small amount of intercellular space. The cells nearest to both epidermes are slightly palisade-like, but the distinction between these layers and the innermost mesophyll is not clearly marked.

There is a space, the **assimilatory cavity,** below each stoma.

The **vascular bundles** are all cut transversely, *i.e.*, they are parallel to one another and to the long axis of the leaf. Each is surrounded by a well-developed sheath of rounded cells termed the " glucose sheath," while the larger ones have also a bundle of sclerenchymatous fibres above and below them.

3. STOMATA

Nicotiana tabacum (Tobacco plant).—Surface (epidermal) section from the lower side of the leaf killed in absolute Alcohol and mounted in Glycerine.

Instead of a section a small portion of the epidermis may be torn off with a needle and mounted in the same way.

The epidermis shows clearly the wavy outline of the cells, which is characteristic of Dicotyledons, and the **stomata,** which can be readily distinguished from the rest of the epidermis by possessing chloroplasts. Each stoma has two sausage-shaped **guard cells** with their concave faces towards each other, enclosing between them the slit or **pore** of the stoma. In preparations this may be closed, unless the section has been rapidly fixed in absolute Alcohol and is examined in the same fluid. The nuclei of the guard cells are visible.

If some of the cells of the spongy mesophyll have remained attached to the epidermis, their irregular shape and arrangement into a loose network may be seen clearly, while it will be noticed that the stomata are usually opposite the intercellular spaces of the network, thus facilitating gaseous diffusion in and out.

Pelargonium zonale (Garden Geranium).—Surface section from the lower surface fixed in absolute Alcohol and mounted in Glycerine.

This may be substituted for *Nicotiana.* The details are almost the same. There are numerous long **hairs** on the epidermis, some bearing oval **secretory cells** at the tips (*cf.* Glands). Each arises from a single epidermal cell.

Iris germanica (Purple Flag Iris).—Surface section from either surface fixed in absolute Alcohol and mounted in Glycerine.

The **epidermal cells** are long and narrow with straight walls and the **stomata** are arranged in longitudinal rows, the stomata in each row alternating with those on each side of it. Each stoma lies at the junction of two epidermal cells and shows two large semicircular **guard cells,** between which lies the slit-like **pore** with a ridge on each side of it.

Note that the guard cells contain chloroplasts while the epidermal cells do not.

Zebrina pendula (Tradescantia zebrina) (or other trailing species).—Surface section from the lower surface fixed in absolute Alcohol and mounted in Glycerine.

The epidermal cells are unusually large, so that it is not difficult to get a surface section including the colourless epidermis only, without any mesophyll. This is what should be aimed at. The **epidermal cells** are cubical with straight walls. The **stomata** are large, the two **guard cells** showing up clearly on account of the chloroplasts they contain. Between them lies the slit, usually seen closed, with an elliptical ridge surrounding it.

There are four **subsidiary cells,** one on each side of the guard cells, and one longer cell across each end. These cells are like the epidermal cells, devoid of chlorophyll.

If a piece of epidermis alone, without adherent mesophyll, is stripped off and dropped immediately into absolute Alcohol and then examined in the same Alcohol, the pores may often be seen fixed in the open condition. They shut if subsequently wetted.

Zea mais (Maize).—Surface section of the leaf parallel to the adaxial surface to show the stomata.

The **guard cells** lie parallel to one another and contain cytoplasm and a nucleus. There is a narrow **subsidiary cell** on each side of the stomata.

Each guard cell is highly thickened in its middle portion and expands into a thin-walled portion at each end, where the guard cells are in contact. The opening and closing of the pore is effected by the expansion and contraction of these thin-walled portions of the cells.

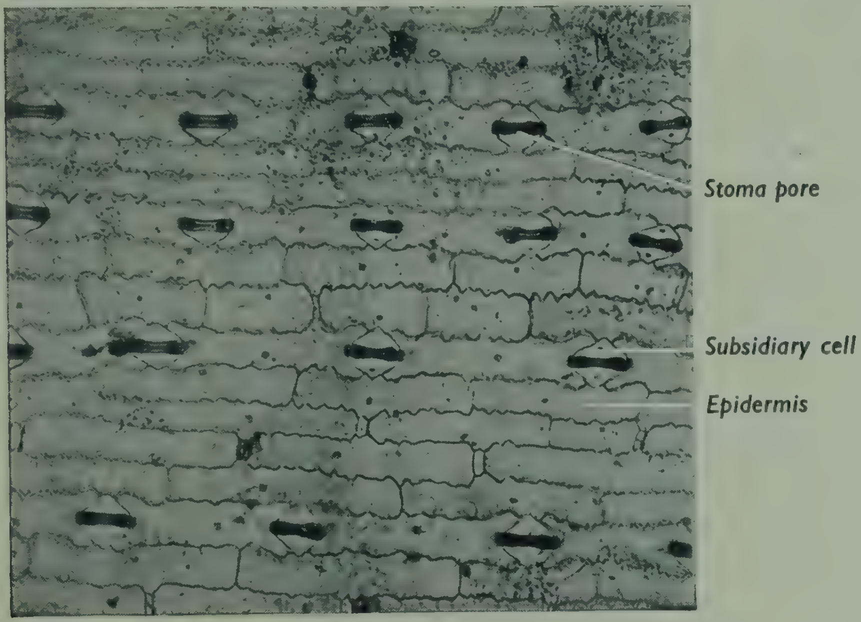

FIG. 51.—*Zea mais*. Superficial section to show stomata.

C. PETIOLES AND LEAF FALL

Phaseolus multiflorus (Scarlet Runner Bean).—Transverse section through the pulvinus; stain with Aniline chloride.

The **vascular tissue** is arranged in a circle as in a stem, which is an unusual but not rare occurrence in dicotyledonous petioles. The vascular ring is surrounded by a zone of five or six layers of large cells which appear to be empty. This probably represents the **pericycle,** but there is no endodermis visible. The **cortex** is broad and its cells are notably large and loose fitting. There is a small-celled **epidermis.**

Prunus lauro-cerasus (Cherry Laurel).—Transverse section about midway up the petiole; stain with Aniline chloride.

The main difference between this type and *Syringa*, the next following, is that the **vascular tissue** is organized into three large bundles instead of a continuous band, but the bundles are arranged in a similar horseshoe curve. Each bundle, moreover, is almost completely sheathed in layers of very thick-walled collenchyma cells. There is no endodermis or pericycle in the petiole, so we cannot separate stele from cortex, but the parenchyma tissues, which may be termed the cortex, are thick-walled. They are loosely aggregated on the upper side and closely packed on the lower side, on which the weight of the leaf exercises some compression.

Syringa vulgaris (Lilac).—Transverse section about the middle of the petiole; stain with Aniline chloride.

The **vascular tissue** is in the form of a horseshoe with incurled ends. The **xylem** is directed towards the adaxial surface and the **phloem** towards the abaxial surface, as in the leaf blade. Xylem and phloem together form a continuous band, not divided into separate vascular bundles.

Beneath the phloem runs an arc of scattered **sclerenchyma fibres.** This zone of fibres, the shape of the vascular zone and the thick-walled tissues which form the two prominent ridges on the upper side of the petiole may be interpreted as features which tend to increase the rigidity of the petiole.

Opposite the two ends of the vascular horseshoe there are two minute bundles, which are **concentric** in structure, the xylem occupying the centre and being more or less completely surrounded by phloem (periphloic structure). These small bundles eventually become the marginal veins of the leaf.

Acer campestre (Maple) or ***A. pseudo-platanus*** (Sycamore).—Longitudinal section through the base of the petiole and adjacent parts of the stem, taken in autumn; unstained to show leaf fall.

A dark-brown band extends across the base of the petiole cutting it off from the cortex of the stem. This is the **phellogen,** which is continuous with the same tissues on the surface of the stem. It forms outwards, a broad band of cork cells, the **abscission layer,** eight to nine cells thick, the layers of which next to the phellogen are conspicuously orange-coloured. Between the cells of the corky abscission layer and the parenchyma of the petiole there is a marked discontinuity, several layers of the cells having been crushed by the pressure of the outwardly growing cork. This is the true plane of severance between the petiole and the stem. The phellogen does not remain active for long, but becomes filled with an opaque brown deposit. If the section passes through a vascular bundle of the petiole a similar deposit may be seen filling the cavities of the tracheids. **Rhomboidal crystals** of Calcium oxalate are very abundant in the cortex of the petiole.

Section 5. Hairs and their Modifications

Primula vulgaris (Primrose).—Surface section from the lower side of the leaf; mount in Glycerine.

Each hair is formed from a row of elongated cells joined end to end. The basal cell is one of the epidermal cells and is shaped like a truncated cone, pointing outwards, and forming a plinth to the hair. The cells taper gradually outwards and the apical cell has a rounded end. Some of the cells may appear collapsed, others contain a yellow deposit of tannin.

Platanus orientalis (Plane Tree).—Surface section or scraping from a young leaf to show compound hairs.

The hairs are multicellular and branched, each having a main axis, with side branches given off in whorls at the " nodes " of the axis. The hair grows from the base and the lower cells contain cytoplasm and a nucleus, while the apical (older) cells are dead and empty. The interlacing hairs form a thick woolly **tomentum** over the whole surface of the leaf, which is shed as the leaf matures, so that when fully grown it is glabrous.

Verbascum thapsus (Mullein).—Shaving of the surface of the woolly leaf.

Each hair is compound, consisting of an axis formed of long cells joined end to end. The axis bears at each " node " a whorl of five to six simple unicellular branches.

Many other woolly leaves will provide similar compound multicellular hairs.

Matthiola incana (Garden Stock).—Surface section from underside of a young leaf.

There is a beautiful tracery of interlacing hairs covering the leaf surface. Each hair is unicellular but branched. There is a short stalked portion growing vertically from the epidermis and giving rise, at its upper end, to a horizontal arrangement of long pointed arms, somewhat like a star fish. Examination with the high power will show that there is one continuous lumen throughout, tapering away to nothing towards the solid tips of the branches.

Many other members of the Cruciferae, *e.g.*, *Arabis*, *Aubrieta*, *Capsella* and *Cheiranthus* have similar hairs to the above though not so much branched.

Phaseolus multiflorus (Scarlet Runner Bean).—Transverse section or epidermal section of the coat of the bean-pod.

Thin-walled unicellular hairs with sharply recurved and thickened points arise from a thick-walled **basal cell.** There are also small multicellular **gland hairs** present between the hooked hairs.

Humulus lupulus (Hop).—Epidermal section of the stem.

Large grapnel hairs with a conical **stalk cell** and two thickened pointed cells, set transversely at its apex, like hooks, are conspicuous.

Rosa sp. (Rose).—Transverse section of the stem through the base of a prickle.

That a prickle is an epidermal outgrowth is shown by the presence of cortex beneath the base of the prickle. The margin where it joins the stem is strengthened by a ring of collenchyma in the cortex and by special thickening of the epidermal cells. The main tissues of the prickle are composed of long thin-walled cells, its rigidity depending entirely on the thickened cells of the outermost layers.

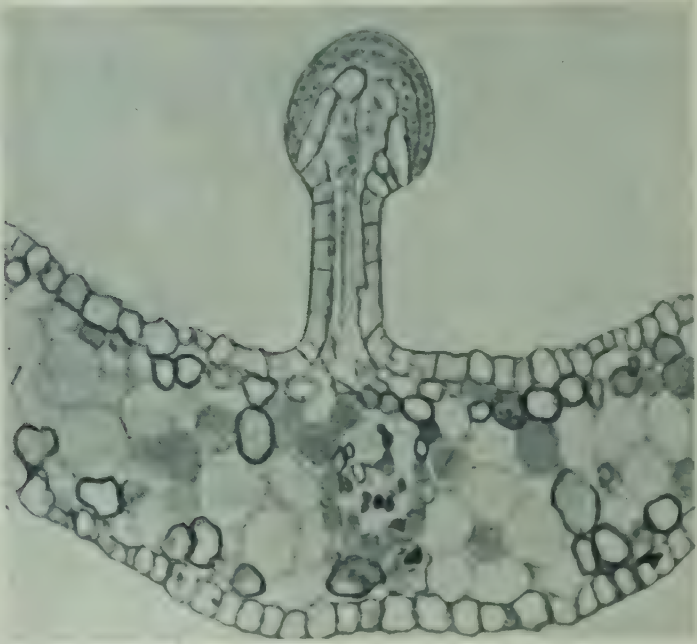

FIG. 52.—*Drosera rotundifolia.* Vertical section of a leaf with gland.

Drosera rotundifolia (Sundew).—Leaf cleared by immersion in Eau de Javelle and mounted whole in Glycerine or, alternatively, cleared in Clove Oil, after dehydration with absolute Alcohol, to show glandular hairs.

There are two types of glandular hairs: (1) Those on the leaf margin, which are the longer of the two, and have long narrow glands at their tips; (2) those on the surface of the leaf blade, which are much shorter and have rounded heads.

In both cases the stalks have narrow vascular strands, ending upwards in a central mass of barred tracheids in the gland (Fig. 52). The hair stalk bears numerous raised stomata, which is unusual and suggests that the "hair" is really a leaf segment.

The gland itself consists of the central tracheid mass, surrounded by a layer of small cells with dense contents. There is a large-celled **epidermis,** in the outer walls of which may be seen pits filled with cytoplasm, which are supposed to be associated with the sensitivity of these hairs to touch.

Pinguicula vulgaris (Butterwort).—Transverse section of the leaf.

There are two kinds of gland hairs on the leaf, both on the upper surface : (1) Glands with a single elongated stalk cell, at the end of which is the gland, composed of sixteen cells, radially arranged, around a central cell ; (2) glands with a very short stalk cell, in a depression of the leaf surface, and a head of eight cells, radially arranged, which lies on the same level as the surface of the leaf.

Both types of hairs are secretory, and both take part in the process of digesting insects.

Urtica dioica (Stinging Nettle).—Section parallel to the leaf surface or the surface of the petiole mounted in Glycerine.

There are both short and long hairs present. The former are unicellular, broad at the base and tapering to a long sharp point. The latter are very much larger and stand upon a pedestal of small cells, which is about three times as high as broad. These latter are the **stinging hairs.** There are also very small glandular hairs present, each with a two-celled head. The stinging hair itself arises from the top of the pedestal already mentioned and is unicellular. The base is much enlarged, forming a flask-shaped portion embedded in the pedestal. The apex is bent over at right angles and enlarged into a small bulb, not a sharp point. The shaft of the hair has a very thick stiff wall, but there is a line of weakness around the base of the apical bulb. When this is roughly touched it therefore breaks off, leaving a lateral opening, like that of a hypodermic needle, at the tip of the hair. This tip enters the skin of the hand, and the pressure of contact forces out the sap from the basal flask-shaped portion, which enters the tissues through the lateral opening carrying the poison with it.

Section 6. Internal Glands

A. OIL GLANDS

Myrtus communis (Myrtle).—Transverse section of leaf mounted in Glycerine.

For general arrangement of the tissues compare *Ilex*, page 228. Large spherical **glands** occur in the mesophyll on both sides of the leaf. Each is a spherical space lined with an **epithelium** of flat tabular cells, which secrete the **oil** (myrtenol), drops of which may be seen in the cavity of the gland. The glands come almost to the surface of the leaf, the epidermal cells above them being reduced in size.

If a whole leaf is held up to the light the glands may be seen as minute whitish points, due to the displacement of the palisade cells where the glands are formed.

Eucalyptus globulus (or other species with transparent dots in the leaves).—Transverse section of the leaf mounted in Glycerine.

The **glands** are large spherical cavities in the mesophyll, which appear empty in the section. Like those of *Myrtus*, they are schizogenous and are lined with an **epithelium** of flattened, thin-walled secretory cells. The upper side of the gland lies almost immediately under the leaf cuticle, being covered only by very small epidermal cells.

Citrus limonia (Lemon).—Section of skin (pericarp); mount in Glycerine.

The **oil sacs** are of various sizes, lying close beneath the very small-celled epidermis. Each sac appears as an empty oval space with a lining of several layers of extremely delicate flattened cells, which usually appear collapsed. At the apex of each gland is a small depression in the epidermis. These oil sacs secrete Lemon oil, which contains 90 per cent. Dextrolimonene. If sections are left for some time lying in Glycerine or if they are cut from the edge of a half fruit that has lain in the air for about half an hour, large numbers of crystal sheaves of the characteristic glucoside **hesperidin** will be seen in the tissues.

Hedera helix (Ivy).—Transverse section of the petiole or young stem stained in Aniline chloride to show secretory canals.

The **canals** are situated in the inner cortex, just outside the zone of pericycle fibres. Look for an oval ring of small, squarish, thin-walled cells, the **epithelium,** surrounding a space, which is the **secretory cavity.** This is a transverse section of a straight longitudinal channel. It is typical of the secretory channels found in most other aromatic plants. In cross section the whole gland appears not much larger than one of the large cortical cells, so it must be looked for carefully. There may be a dozen or more of them more or less equally spaced around the section. The secretory channels contain a colloidal solution of a terpinoid saponin called Hederin.

B. LATEX

Taraxacum officinale (Dandelion).—Tangential longitudinal section of the root, stained in alcoholic tincture of Alkannin, washed quickly in water and mounted in Glycerine.

The phloem is largely parenchymatous, with thin-walled cells, and the Alkannin stains dull red the contents of a number of long, thin, and apparently jointed **latex vessels,** which run longitudinally in irregular lines through the parenchyma. The vessels usually occur together in small groups, and they both branch and anastomose. The latex is granular, and consists largely of resin and tannin material precipitated in the cells.

Inulin (p. 42) is also visible if the material has been preserved in strong Alcohol.

Euphorbia splendens.—Longitudinal section of the stem stained with a tincture of Alkannin or else stained with Iodine to show up the starch grains in the latex tubes and mounted in Glycerine.

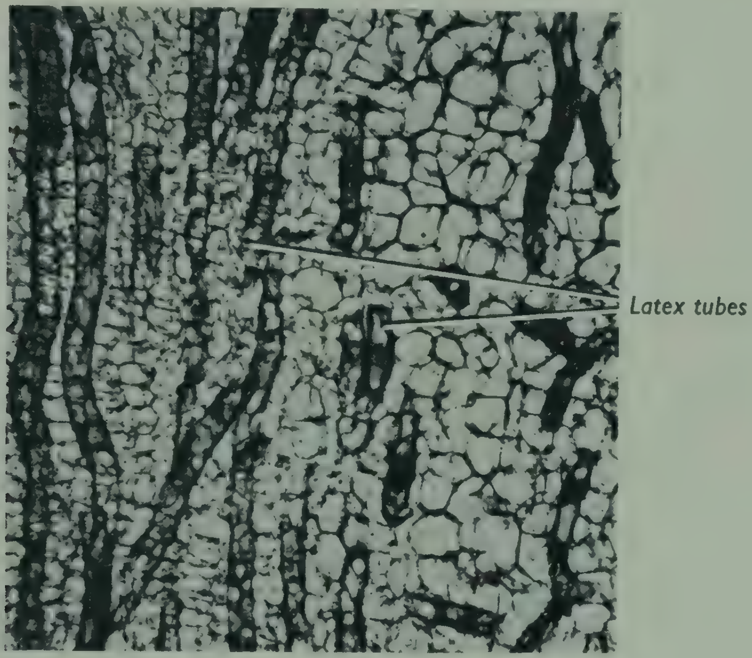

FIG. 53.—*Euphorbia splendens.* Longitudinal section of the stem with latex tubes.

In the cortex are broad **latex tubes,** winding irregularly through the tissues, branching and anastomosing and devoid of cross walls (Fig. 53). The contents are stained purplish-red by Alkannin. The tube walls are much thicker than those of the cortex cells. The latex contents appear densely granular and contain numerous **starch grains** of a unique dumb-bell shape, quite unlike those in the cortical cells.

Euphorbia splendens is a xerophyte and has a strong **periderm** composed of a large number of layers of collapsed and tightly compressed cells with suberized walls.

PART FIVE

SEXUAL REPRODUCTION OF ANGIOSPERMS

Section 1. The Morphology and Anatomy of Flowers

A. FLORAL PARTS AND SYMMETRY

Ranunculus acris or other species (Buttercup).—The flower is a "perfect" type, in the sense that all the categories of parts are represented. The stalk, or **pedicel,** ends at the top in the **floral receptacle,** a narrow conical or club-shaped portion to which the parts of the flower are attached.

Lowest on the receptacle come the hairy green **sepals** forming the **calyx.** Next above these are the **petals** (**corolla**) at the base of each being a minute pocket which contains a **nectary.** Sepals and petals are all free from one another (**aposepalous** and **apopetalous**). Above the petals come the **stamens** (**androecium**), each consisting of a delicate stalk (**filament**) and a head (**anther**) of approximately equal length. The anthers curve inwards and open, when ripe, on the outer side (**extrorse**).

Uppermost are the **carpels** (**gynoecium**), forming the heart of the flower. Each carpel consists of a minute sac (**ovary**) containing a single **ovule.** At the upper end the ovary is extended into a short stalk (**style**) terminating in a roughened knob, the **stigma.** All the carpels are free from each other, *i.e.*, they are **apocarpous.** Since the gynoecium stands above the other parts, the flower is therefore **hypogynous.**

The flower is symmetrical about its central point, and it is therefore **actinomorphic.**

Caltha palustris (Kingcup).—The flower resembles *Ranunculus* in its essential structure, with the following differences: there are no petals, the five or more **sepals,** which make up the **perianth,** being all yellow and **petaloid** in appearance. There may be up to a hundred spirally arranged **stamens**; the **carpels** form a group of five or more and develop into many-seeded follicles. Each seed has a flattened **aril,** developed at the **chalazal** end, which is an outgrowth of the outer integument. See page 269 for seed dispersal.

Helleborus niger (Christmas Rose).—Resembles *Caltha*, except that the petals are represented by five tubular **nectaries,** which are greenish in colour and alternate with the white **sepals.**

Prunus cerasus (Garden Cherry) provides an example of a flower that, like the Buttercup, is **actinomorphic** and **polypetalous.** In this case, however, the floral receptacle makes a cup round the solitary carpel. The other parts of the flower are attached to the edge of this cup and instead, therefore, of being below the gynoecium (**hypogynous**) they stand round about it, or are **perigynous.** *Prunus domestica* (Plum) shows a similar structure.

Rosa (any species) may be taken as an example of more extreme **perigyny,** where the receptacular cup is more like a flask, being almost closed over the carpels, of which only the styles protrude through the opening.

Pyrus malus (Apple) or ***P. communis*** (Pear).—These are also actinomorphic polypetalous flowers, but in them the receptacle has entirely closed over the carpels, which are therefore only to be found embedded inside a mass of receptacle tissue, forming the core of five carpels, in the flesh of the fruit. Thus the other parts of the flower are here carried above the gynoecium, and the flower is **epigynous.** As the receptacle forms the greater part of the fruit the latter is classed as a **pseudocarp** (see p. 266).

Primula vulgaris (Primrose).—These flowers are radially symmetrical or **actinomorphic** like those of the Buttercup. They differ radically from the latter, however, in that the petals are joined together to form a narrow tube surrounding the stamens and carpels. They are called **sympetalous.** Each petal has two portions, one the narrow lower portion called the **claw,** which forms part of the floral tube, and an upper expanded portion or **limb.**

The **stamens** are joined to the perianth tube (**epipetalous**) instead of independently to the floral receptacle. The **carpels** number five, but they are closely united (**syncarpous**) and the septa have disappeared, so that only one **loculus** or seed chamber is left. The **placenta** rises from the base of this loculus, and is covered with **ovules** (**free central placentation**).

For details of the pollination mechanism of *Primula* see Section 2, page 251.

Viola odorata, V. canina, or other species (Violet).—The **perianth segments** are all free, but the flower is symmetrical only about one plane, that running vertically through the centre. It is only thus that the flower can be divided into two equivalent halves. Such flowers are called **zygomorphic.** The two back petals are narrow and bent backwards, the two side petals have each a tuft of white hairs, while the lower, largest petal is produced backwards into a hollow **spur.**

For further details of the flower and the pollination mechanism see page 275.

Digitalis purpurea (Foxglove).—The flower is **zygomorphic,** like *Viola*, but less markedly so, the lower petal being only somewhat bulged downwards, not spurred. The petals are, however, all joined into a tube **(sympetalous)**. So also are the sepals, only the tips of which are free **(synsepalous)**.

Lamium album (White Dead-Nettle) is another common example of the same type of symmetry, though here the difference between the lower and upper lips of the floral tube is considerably greater than in *Digitalis*.

B. FLORAL DEVELOPMENT

Chrysanthemum leucanthemum (Ox-Eyed Daisy).—Flower heads about 5 to 6 mm. across will show interesting early stages in the development of floral parts. Cut median longitudinal sections, stain in Aniline chloride and mount several in Glycerine.

The flowers develop in **acropetal succession,** the youngest being near the centre, or apex, of the capitulum (Fig. 54). These youngest flowers will show little except the young **petals** (with a sort of crown of large cells on each, with finely striated walls) arched over a group of rounded humps of tissue which are the **stamen rudiments.** The lower part of each flower is still a solid mass of tissue, the **receptacle,** and the stamen rudiments are quite undifferentiated and consist entirely of meristematic parenchyma. In the outer florets the **archesporium** is plainly distinguishable in the stamens by the denser appearance of its cells. In some of the older flowers the beginning of **carpel formation** is shown by the development of an invagination, in the form of a deep central pit or sinus running down into the solid tissue of the flower base. This sinus deepens, while its margin grows upwards between the stamens and forms the **style.**

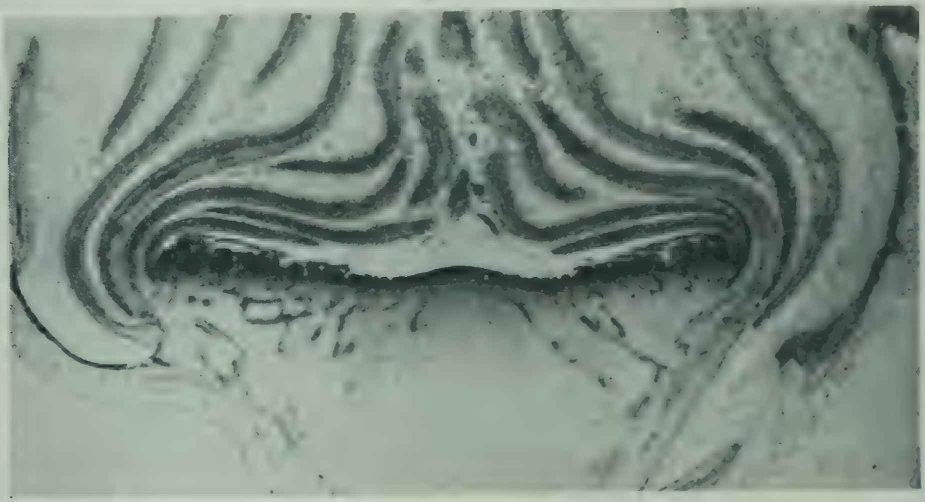

FIG. 54.—*Helianthus rigidus.* Longitudinal section of the young capitulum.

The gynoecium is thus seen to be literally sunk into the receptacle, the flower being **epigynous.** The sinus widens at the bottom and becomes flask-shaped, and in this cavity, which is the **ovary,** there rapidly develops from the base a single ovule, which turns over and becomes **anatropous.**

In flowers at this stage the commencement of the **pappus** can also be seen as small outgrowths at the base of the petals, external to the flower.

C. STRUCTURE OF THE ANDROECIUM

1. ANTHER

> ***Lilium,*** any species (Lily).—The Lily makes excellent floral material because of the large size of its parts, but other Liliaceous flowers, *e.g.*, Tulip or Solomon's Seal, may be used successfully for the same purpose. Make transverse sections through the **anthers** in a bud, before opening and while it is still under 2 in. long. Cut the entire bud transversely, beginning at the tip, and select sections which pass through the anthers. If the material is fresh these should be stained with acetic Methyl Green (see p. 22) and mounted in Glycerine. Preserved or fresh material may be stained with Ehrlich's Haematoxylin, counterstained with Erythrosin (see p. 17) and also mounted in Glycerine. Smear preparations of young anthers may also be tried, see Part I, page 22.

At this early stage the pollen will probably still be in the mother-cell condition or at some phase of meiosis, and the large nuclei are readily seen. Meiosis is, however, passed through quickly, and the exact stage found will vary with the species of Lily used and the weather at the time. The earliest stage found is likely to be one in which the **mother cells** have rounded off and are surrounded by the disintegrating cells of the **tapetum,** much of the cytoplasm of which has migrated among the developing mother cells. At this stage the anther wall is not fully differentiated, and the cells contain numerous starch grains.

During maturation various phases of the heterotypic or homotypic division may be found, ending with the formation of a **tetrad** of cells which become the actual **pollen grains.** The exact phase found may be identified by reference to one of the works on Cytology, such as Sharp's " Introduction to Cytology."

The later stages will show completed and cuticularized **pollen grains,** with a granulated pattern on the outside of the **cuticularized extine.** If microtome sections are available they will probably show that each mature grain contains two nuclei (Fig. 55). One is a rounded nucleus, the **vegetative nucleus,** *i.e.*, the nucleus of the pollen grain as a cell. This later becomes the **tube nucleus** in the growing pollen tube. The second nucleus is oval, and is separated from the rest of the cell by a delicate wall, forming a sac round it. This is the **generative nucleus** which produces the male nuclei. It is hardly probable that such details will be observed in hand sections, but by good fortune something of the cell structure of the mature grains may be seen, or prepared slides may be purchased.

At the latest stage the tapetum is reduced to a mere shell of cytoplasm round the **loculus,** with numerous nuclei lying free in it, and the anther wall shows the gradual disappearance of the protoplasm in the **middle layer** and the appearance on its walls of cuticularized bands which assist in the dehiscence of the anther.

2. TYPES OF POLLEN GRAINS

The form and appearance of pollen grains are extremely varied, but those belonging to the same genus and even to the same family of plants show recognizable resemblances, so that the systematic affinities of a plant may often be judged by a study of the pollen alone. The following are a few examples which show characters common to one or other of the important families.

Pollen grains may be examined in the air-dry or in the fresh states. Fresh grains from an anther are treated with absolute Alcohol, lightly stained with Basic Magenta and mounted in Glycerine jelly.

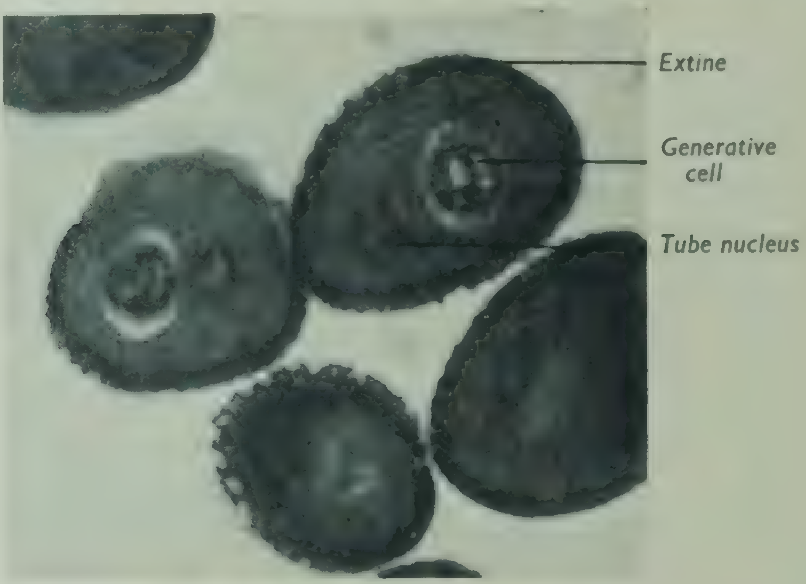

FIG. 55.—*Lilium* sp. Sections of mature pollen grains showing the nuclei.

***Malva* sp.** (Malvaceae).—The grains are unusually large (about 0·1 mm. in diameter), spherical and of a bright golden-yellow, the colour being in the **extine.** The extine is covered with sharp spines, while here and there is a larger spine which arises from a small disc of extine standing out from the general surface level. These discs are detachable, and are pushed out by the swelling of the **intine** at the germination of the grain, providing exit pores for the pollen tubes.

***Oenothera* sp.** (Evening Primrose).—The grains are three-sided pyramids, with a rounded extension at each angle, the whole densely filled with cytoplasm. The round appendages have extra thick walls, but the general surface is practically devoid of special markings, beyond a slightly granular appearance. Four-angled grains occur in certain polyploid races.

Acacia dealbata (The "Mimosa" of commerce).—The grains are compound and consist of packets of sixteen small, angular cells, which are arranged in a flat plate, two cells thick in the middle. Other species of *Acacia* possess similar grains but the number and arrangement of the component cells varies. On germination each cell produces an independent pollen tube.

Lilium sp.—The grains are oval, yellow or brown in colour, with a fine network of cuticular ridges on the extine and usually many small adherent drops of coloured oil. A deep groove runs down one side, and it is here that the extine splits when the grains germinate on the stigma.

Arrhenatherum elatius (False Oat Grass).—The interesting type of grass pollen can be well seen in this very common grass. The grains are very broadly oval, nearly spherical, and the extine is very fine granular, with much oily material adhering to it. At one end the extine is thickened, and in the middle of this is a small circular ridge of cuticle surrounding a thin spot which is the germination pore. This pore is covered only by the intine, in the middle of which lies a small protective button of extine. Inside the cell the starch grains lie in a mass at the end nearest the pore, and this, together with the extra thickness of wall at that end, so balance the grain that it falls through the air, from stamen to stigma, invariably with this end downwards. The pore, therefore, lies next to the stigma surface when the grain comes to rest on it.

D. STRUCTURE OF THE GYNOECIUM

1. OVARY AND OVULE

Scilla non-scripta (Bluebell).—Transverse section of ovary taken during flowering.

The section shows three **loculi** or carpel cavities, (*i.e.*, the ovary is compound), containing **ovules** which are attached to the central **axis** (**axile placentation**). The outer wall of each loculus is the **carpel wall,** which later becomes the **pericarp** of the fruit, and the division walls between them are the **septa.**

The ovules are seen in pairs in each loculus, representing two longitudinal rows, and each is attached by a short stalk, the **funicle,** which is fused to one side of the ovule. The **micropyle** of the ovule is turned in towards the central axis of the ovary (**anatropous** position). If a number of sections are cut and mounted together one will probably be found in which the structure of the ovule may be seen.

The two **integuments** are united over the most part of the ovule and can only be seen as separate layers at the micropyle. The latter is, in this case, not an *open* orifice, but only a narrow channel through the integuments, the sides being closely appressed to each other.

The **nucellus** is a thin layer, not much more than one cell thick, and it contains a very large **embryo sac** in which some of the nuclei, *e.g.*, oosphere, synergidae, antipodals or endosperm nuclei, may be distinguishable with careful focusing.

If the ovary has been collected when the petals were withering, early stages of embryo formation (see below) will be found.

2. DEVELOPMENT OF THE OVULE

> ***Fuchsia riccartoni.***—Transverse sections of the ovary. The details of this process require microtome sections, but the following important stages can be easily observed in hand sections. Numerous sections, not too thin, should be cut, and should be well *cleared* in Glycerine or Eau de Javelle.

1. In buds which, together with the ovary, measure 1 cm. long. There are four **loculi,** each showing two young **ovules,** which at this stage are only masses of parenchyma cells, curved and with rounded ends, but showing no differentiation of parts. Note, in addition, in the wall of the ovary the colourless lining of the loculi (**endocarp** layer), green **mesocarp** containing vascular bundles and masses of raphides, and the colourless or reddish **epicarp** or outer layer bearing club-shaped glandular hairs.

2. In buds measuring 1·5 cm. over all. The **ovule** shows a stalk portion, the **funicle,** and a more or less globular apex, the beginning of the **nucellus,** round which appears a collar which is the earliest phase of the **inner integument.** This will grow and cover over the nucellus leaving only the **micropyle** opening.

3. In buds measuring 1·8 cm. over all. The nucellus has turned down towards the foot of the funicle into the **anatropous** position. The inner integument has almost completely enclosed it, and the beginnings of the **outer integument** may be seen arising from the side next to the funicle. In the latter a vascular bundle is already differentiated.

4. In buds measuring 2 cm. over all. The outer integument is almost completed, and the young **embryo sac** may be seen in the nucellus in fortunate sections.

> ***Caltha palustris*** (Kingcup).—The young carpels of this flower may by cut transversely by hand without much difficulty and, if a number of sections are examined, one or more will be found to show good views of the embryo sac in the ovule. Choose a fully opened flower before the stamens have been shed. Mount sections in Glycerine.

The ovules are attached to the carpel wall in one line, but alternating right and left. There are two parallel vascular bundles along this, the ventral side of the carpel, and the ovules are supplied alternately from one or the other of these bundles. They may be regarded, therefore, as forming two rows, which are united into one. The **funicle** may therefore be either to the right or the left of the particular ovule in your section. The ovules are **anatropous,** being turned over on the funicle, so that the micropyle faces the carpel wall. The pollen tube (see p. 253) penetrates the carpel wall tissues, and the anatropous position of the micropyle no doubt facilitates its entry into the ovule. The funicle has a single vascular bundle which ends at the **chalaza** or hinder end of the ovule. The chalaza is usually well developed in *Caltha* and later is transformed into a spongy mass which helps to float the ripe seed.

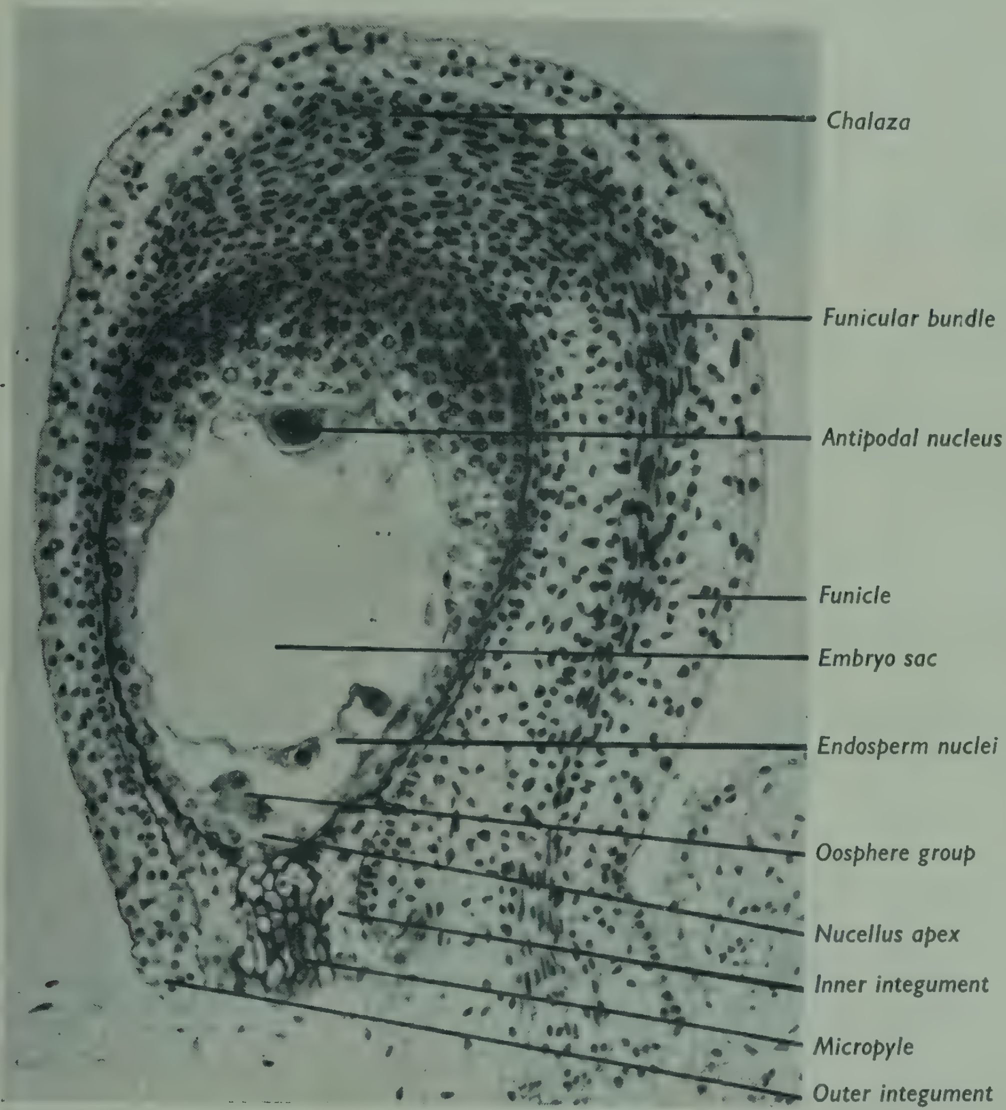
Chalaza
Funicular bundle
Antipodal nucleus
Funicle
Embryo sac
Endosperm nuclei
Oosphere group
Nucellus apex
Inner integument
Micropyle
Outer integument

FIG. 56.—*Narcissus majalis.* Longitudinal section of the ovule and embryo sac.

There are two **integuments** or coats to the ovule, tightly enclosing the **nucellus** which is two to four cells in thickness. The **micropyle** is not actually an open canal but only a channel between the tips of the inner integuments which are in contact with each other. The **embryo sac** occupies the greater part of the nucellus. It is so large that it is improbable that all its nuclei will be seen in one section (Fig. 56). They are arranged thus: at the micropylar end two **synergids** and the **oosphere nucleus** in a group of three together, the synergids being nearest the micropyle. Two

endosperm nuclei, in the middle of the sac and usually close together, and the three **antipodals,** particularly large and prominent in this genus, lying in a dense mass of cytoplasm and separated from each other by delicate membranes. No endosperm tissue is formed until after fertilization.

> ***Lilium*** any garden species. Sections may be cut transversely from a gynoecium, about 2·5 mm. in diameter, which should show intermediate stages in the maturation of the embryo sac. Sections may be stained in Ehrlich's Haematoxylin and then washed in very dilute alkali before mounting.

The Lily is so often used as a type in the study of the floral organs, on account of the large size of the parts, that it is important to point out that the embryo-sac development does not follow the normal course. There are four successive divisions of the original nucleus, the first two of which constitute a meiosis. In these divisions it is interesting to note that there is the beginning of cell-plate formation between the daughter nuclei at the telophase. These cell plates, however, are only vestigial and soon disappear, since they represent the partial separation of the megaspore mother cell into four megaspores, a process which is not completed in *Lilium*, since the embryo sac is formed directly from the megaspore mother cell and not from a megaspore (Fig. 57).

There is a further peculiarity in the behaviour of the nuclei ; after the meiotic divisions the four nuclei arrange themselves so that one remains at the micropylar end of the embryo sac while the other three pass to the chalazal end. These latter nuclei fuse together to form a single triploid nucleus, after which both nuclei divide again. Thus two monoploid and two triploid nuclei are formed. A further division of each nucleus results in the embryo sac containing four monoploid nuclei and four triploid nuclei, the monoploid ones lying near the micropyle and the triploid ones at the chalazal end. One monoploid and one triploid nucleus now pass to the centre of the sac and there fuse, to form the primary endosperm nucleus. Hence when double fertilization occurs there is a fusion between a monoploid nucleus and one male nucleus, and at the same time a fusion of a tetraploid nucleus and the other male nucleus.

E. TYPES OF INFLORESCENCES

The flowers are often segregated on a special part of the plant in which the foliage and the branching differs from those in the vegetative shoots. These specialised flowering parts are called **inflorescences,** and they display characteristics which have led to the recognition and naming of a number of types.[1]

The examples given below are all easily found, but once any type has

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[1] No truly exact classification of inflorescences has yet been formulated. Exceptions exist to every type which has been suggested. The system here given makes no claim to finality, but is very generally used.

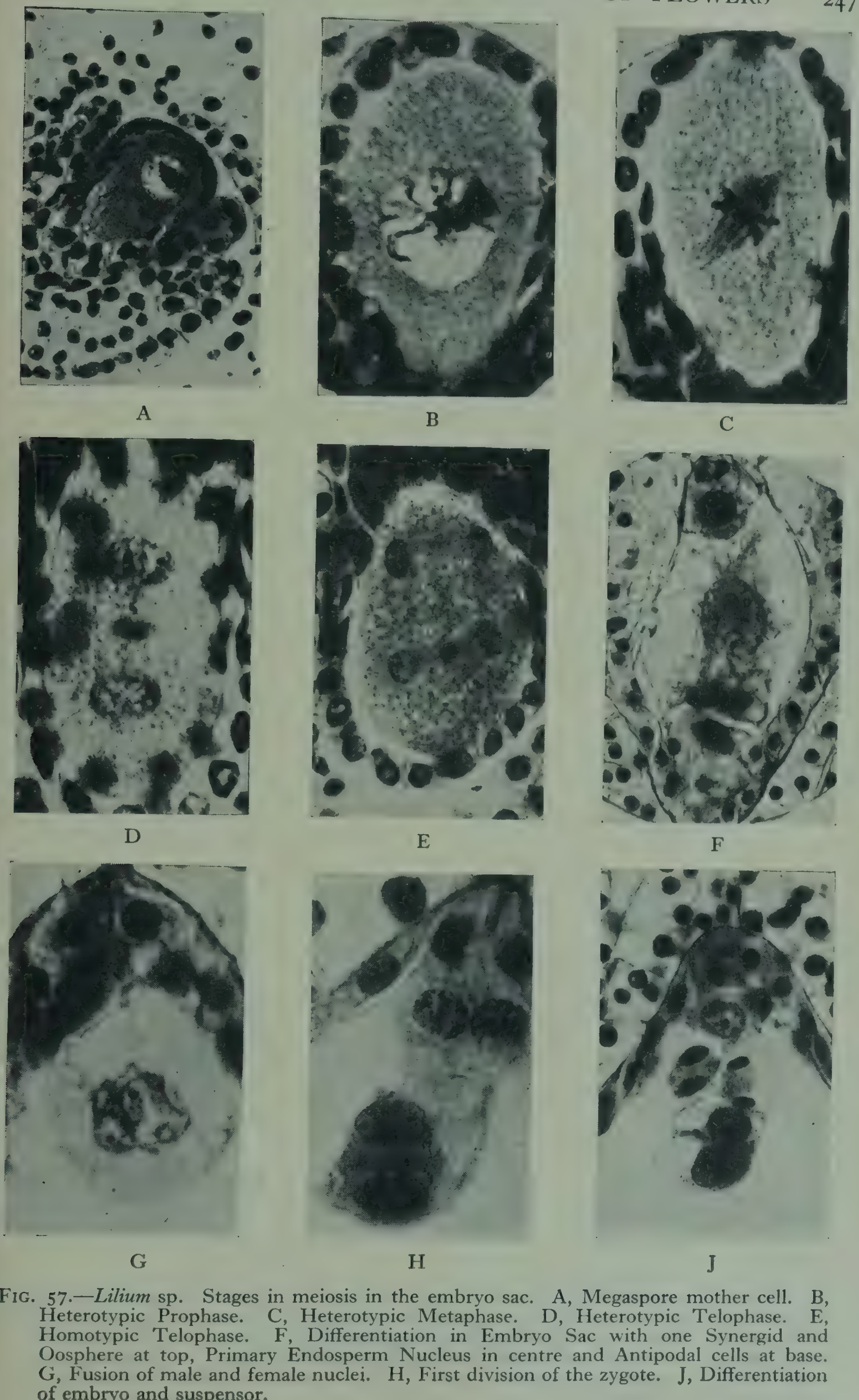

FIG. 57.—*Lilium* sp. Stages in meiosis in the embryo sac. A, Megaspore mother cell. B, Heterotypic Prophase. C, Heterotypic Metaphase. D, Heterotypic Telophase. E, Homotypic Telophase. F, Differentiation in Embryo Sac with one Synergid and Oosphere at top, Primary Endosperm Nucleus in centre and Antipodal cells at base. G, Fusion of male and female nuclei. H, First division of the zygote. J, Differentiation of embryo and suspensor.

been understood and recognized, many other suitable examples will readily present themselves.

1. **Racemose Types.**—Flowers in an indeterminate sequence, *i.e.*, the apical growing-point remains active and the youngest flowers are therefore nearest to the apex.
    - (*a*) **Raceme.**—Flowers with pedicels, arranged in a simple acropetal succession. *Convallaria*, *Prunus*, *Laburnum*.
    - (*b*) **Spike.**—Flowers arranged as above, but sessile. *Plantago*, *Salix*.
    - (*c*) **Umbel.**—Flowers with pedicels, in acropetal succession, but springing from one point only, at the apex of a stalk, *i.e.*, forming a condensed raceme. Seldom simple, more usually compound, that is an umbel of umbels. *Anthriscus*, *Heracleum* (both compound), *Pyrus* (simple).
    - (*d*) **Corymb.**—The flowers originate on a main axis as in a raceme, but the lower flower stalks are so much longer than the upper ones that the flowers form a flat head of greatly enhanced conspicuousness. *Iberis* (Candytuft). In this example it should be noticed that the corollas are irregular, the outer petals being enlarged.
    - (*e*) **Capitulum.**—Flowers sessile on a flattened receptacle, forming a discoid or sometimes a globular head, surrounded by bracts, *i.e.*, a condensed spike. *Taraxacum*, *Bellis*.
    - (*f*) **Panicle.**—A compound raceme, at least in its lower parts. *Poa* and many other grasses.
2. **Cymose Types.**—Flowers in a determinate sequence, *i.e.*, the inflorescence has a terminal flower, which is the oldest, the younger flowers coming from the axils of lower bracts, but sometimes overtopping the main axis.
    - (*a*) **Dichasium.**—Lateral flowers paired, on opposite sides of the axis. *Stellaria*, *Cerastium*, *Euonymus*.
    - (*b*) **Monochasium.**[1]—Lateral flowers single. *Myosotis*, *Sedum*.
3. **Bracts and Bracteoles.**—The modified leaves of the inflorescence are known as **bracts**. There may be a single large bract subtending the whole inflorescence, as in sedges and some grasses, while normally each individual flower also springs from a bract axil. Where flowers are agglomerated together, as in an umbel or a capitulum, the bracts are usually also associated together to form a common group or **involucre** round the inflorescence.

    In a compound umbel there may be an involucre to the main umbel and an **involucel** to each small **umbellule**.

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[1] Various terms have been used to describe apparently different forms of monochasium, in particular **scorpioid**, for the case where successive flowers alternate on opposite sides of the axis and **helicoid**, for the case where they are produced always on the same side. It has, however, long been understood that these distinctions cannot always be realized in practice and that the terms are more theoretical than practical.

Sometimes, *e.g.*, *Viola*, small bracts (**bracteoles**) occur on the flower stalk itself, above the bract which subtends the flower. These are presumed to be the bracts of suppressed lateral flowers, so that *Viola*, for example, is said to have an inflorescence of a one-flowered cyme, the actual terminal flower being the only one developed. Similar bracteoles are found in *Scilla hispanica* and other species of Bluebell.

Section 2. Pollination

A. ENTOMOPHILY

Erica cinerea (Heather) or ***E. tetralix*** (Cross-Leaved Heath).—The bell-shaped flower is pendulous and is contracted slightly at the mouth, while the entrance is almost closed by the style and the ring of anthers. The **style** protrudes farthest and ends in a blackish, sticky **stigma.** The **anthers** open by pores, which are obliquely placed on the side of the anther nearest the style. The pollen is therefore retained so long as the anther remains closely pressed to the style. At the back of each anther, however, there are two divergent **appendages,** and the only way for a bee's proboscis to reach the nectar, which is round the base of the ovary, is between one or other of these pairs of appendages. When they are disturbed the anther is moved out of position and a shower of pollen falls on to the bee. In visiting another flower this pollen-dusted area will probably come into contact with the protruding stigma and so produce cross-pollination.

Some pollen always falls on the edge of the stigma and adheres to it, so that self-pollination is not excluded.

Chrysanthemum leucanthemum or ***C. maximum*** (Ox-Eyed Daisy).—The individual florets are **protandrous.** The pollen is shed inside the tube formed by the **joined anthers.** The developing **style** grows upwards into this tube, pushing the pollen before it, until it appears as a loose heap at the top of the floret.

As the capitulum is a condensed raceme the development of the florets is centripetal, all those at equal distances from the centre (apex) being in the same stage simultaneously. Thus in young capitula a conspicuous ring of florets may be seen all shedding pollen at the same time. This pollen is carried off on the bodies of flies which frequent the flowers, and by the time it is nearly all gone the wave of development has passed inwards, and the flowers which have been shedding pollen enter the second, or female stage, when the two stigma lobes open out and become receptive. These stigmas pick up pollen, which flies carry from younger florets, thus being cross-pollinated. They also become strongly incurved so that they may also be, in part, self-pollinated by the remainder of the pollen from their own florets.

Arum maculatum (Cuckoo Pint).—Pollination is carried out by small dipterous flies of the genus *Psychoda*, which may sometimes be found in large numbers inside the base of the **spathe.** They are attracted by the carrion odour of the **spadix** in its early stages and by the warmth produced by the rapid respiration of the starch with which the spadix is filled. They pass down into the expanded base of the spathe, past the downward-pointing appendages of the **sterile florets.** Here they are imprisoned, since these appendages prevent a return journey.

The inflorescence is strongly **protogynous,** so that the first insect visitors find the stigmas ready to receive any pollen the flies may bring from other flowers. A certain amount of nectar is secreted by the ovarial surfaces, enough to keep alive a certain number of the visitors, who are obliged to stay until the stamens have ripened. When this happens pollen is extruded apically in sticky masses which are soon smeared over the flies. The barrier of sterile appendages now withers and the prisoners are free to depart to younger and still attractive inflorescences, carrying this load of pollen with them.

Sarothamnus scoparius (Broom).—The flowers do not form nectar, but they are visited regularly by bees, presumably for the pollen. The mechanism is explosive. The **carinal** or keel petals are joined along both upper and lower margins, so that in the young flowers the sexual parts are completely enclosed. The bee alights on the **alae** and presses them downwards. They in turn press down on the carina, and the upper margin begins to split from behind forwards. When the split has reached half-way the five shorter stamens spring out and hit the underside of the bee. They have already dehisced, so that pollen is scattered on the bee's body. When the split reaches the front of the carina the five long stamens spring out with greater violence than the others. The **style** so far has lain against the outer end of the carinal pouch. When released it curls downwards in a complete loop, and as the split comes round to where its tip now lies it is released a second time and springs up hitting the bee on its back, carrying with it some of the pollen on its expanded end. It may thus give pollen as well as pick it up. The style remains curled round after the flower has exploded, so that it may be cross-pollinated by a second visitor.

Berberis darwini, B. stenophylla or other species.—The nectaries lie at the base of the petals and are closely covered by the lower portion of the stamen filaments. The nectar, therefore, tends to collect between the stamen bases and the ovary. The latter stands upright, like a little column, in the centre of the flower, and on the top of it there is a disc-like stigma with a receptive edge to which pollen adheres. The lower portion of the inner surface of the stamen filament is irritable to touch, and the bees which visit the flower inevitably touch this sensitive area when sucking the nectar. The result is a sharp inward movement of the stamen, bringing the anther against the bee's proboscis or head. After a few visits the bee will probably have received pollen on all sides of its proboscis, so that it inevitably rubs some off on to the receptive edge of the stigma in each flower visited thereafter.

Salvia dichroa or other species.—Nectar is secreted by a coloured **nectary** which surrounds the base of the ovary. The **corolla** is very strongly two-lipped, the upper lip forming a curved hood and the lower lip forming a landing platform for the bees. The corolla is so contracted at the mouth that only a small space is left for the insertion of a proboscis.

The long **style** lies in the upper hood and is hidden during the first, the male phase, of the flower. There are only two **stamens,** and they are very peculiar. Each has a very short filament, attached to a lateral petal, just inside the mouth of the flower. On this filament is carried a long curved connective, one limb of which reaches up into the hood and there bears two pollen sacs, constituting half of an anther. The other limb extends downwards and is sterile, but it is expanded into a curved plate somewhat like a shoulder-blade in shape, which is the other half anther. The two plates, one belonging to each stamen, lie side by side, and are joined at their extremities to form a kind of yoke which fits over the bee's proboscis. The insect is obliged, by the position of this yoke in the entrance to the flower, to pass his proboscis through it to get at the honey, and the pressure thus exerted on the stamens brings down the fertile lobes from above by a bent-lever action, thus rubbing pollen on the bee's back. This action twists the stamen filaments so that when the bee withdraws the stamens spring back into their original positions.

After the pollen has been nearly all removed the style matures and elongates, coming out of the hood and expanding the two **stigmatic lobes,** ready for pollination.

Some chance of self-pollination exists, as pollen production and stigmatic reception overlap in time.

Primula vulgaris (Primrose).—The flowers of many species of *Primula* show an interesting **dimorphism.** The two forms are known respectively by the colloquial names of " pin-eyed " and " thrum-eyed " (" thrum " is an old word for a wool-spinner's bobbin). More formally they are called long-styled and short-styled forms. In the former case the style reaches to the top of the floral tube, so that the stigma appears like a pin's head at the opening in the centre of the flower. In this type the stamens are short and are to be found half-way down the inside of the corolla tube. The short-styled form is just the opposite. The style reaches no higher than the level of the anthers in the other type, while the anthers here are seen as a group of five at the opening of the tube. Usually each plant bears only flowers of one or other form.

Charles Darwin in 1862 showed by experiment that the advantages of cross-pollination, *i.e.*, more and better seed, better germination and stronger offspring, were only to be obtained by crossing the two different forms (legitimate pollination); the crossing of two flowers of the same form, even when on different plants, was " illegitimate " and gave poorer results.

In the pin-eyed type all parts of the gynoecium are bigger and better developed, but in the thrum-eyed type the androecium is the stronger. This may be regarded as an approach towards the unisexual state.

Only long-tongued insects such as bees have a proboscis long enough to reach the bottom of the corolla tube for the honey secreted there. In the thrum-eyed flower the pollen will then be deposited at the base of the proboscis near the insect's head. Pollen in this position can only reach the stigma in a long-styled flower, and vice versa with the anthers of the long-styled form, thus ensuring automatically a legitimate pollination. This is, moreover, assisted by the protandry of the flower, that is to say, stamens ripen before stigmas.

B. ANEMOPHILY

Plantago lanceolata and ***P. media*** (Plantain).—The first of these two species is almost entirely **anemophilous** and depends on **protogyny** to ensure cross-pollination between flowers of the same spike. The flowers at the base of the short spike mature first, and the **style** is thrust out from inside the still closed bud. When the male stage is reached the perianth segments fold back and the long **stamen filaments** grow out with " versatile " easily shaken **anthers** containing very loose powdery pollen. The style now withers, but the pollen reaches the styles of flowers farther up the spike which are still in the female stage. Finally, the stamens wither without falling off, so that the zone of ripe stamens which advances up the spike is followed by a zone covered by the withered remains of stamens from the earlier flowers.

P. media, on the other hand, has a longer cylindrical spike. Flowers open in the same order as in *P. lanceolata*, but the stamen filaments are usually lilac-coloured, the anthers are not versatile and the pollen is adhesive. It is visited regularly by humble bees for the pollen.

Quercus robur (Oak).—In the Oak there are male and female inflorescences borne separately on the same trees. The **male flowers** are grouped in delicate pendulous catkins emerging from the axils of the scale leaves at the base of the current year's shoots, while the **female flowers** are short and squat structures borne in the axils of upper leaves on the same shoots.

The male catkin bears about a score of flowers each consisting of a small group of **stamens** surrounded by membranous bracts. The female has numerous, very closely set, spirally arranged bracts round its base, and a single central **tricarpellary ovary** with inconspicuous perianth segments, only the three **stigmas** protruding at the top of the flower.

The male catkins are easily swayed by the wind, releasing the pollen. They ripen before the female flowers so that cross-pollination is the rule.

Pollination is similar in other catkin-bearing trees, with the exception of *Salix*, which produces nectar and is entomophilous.

Arrhenatherum elatius (False Oat Grass).—The anthers are **versatile** and hang out from the spikelets on long, delicate filaments. The flowers are **protandrous,** so that when the upper flowers of the spikelets are shedding pollen the lower flowers have expanded their large, feathery stigmas. Pollen is usually shed in the early morning and some part of it may drop directly on to the stigmas of the flowers below; the rest is distributed by wind to the stigmas of neighbouring spikelets.

This type of pollination is characteristic of most common Grasses.

Zea mais (Maize).—The male and female flowers are in distinct inflorescences on the same plant. The males form a large terminal panicle but the female inflorescences are axillary and are enclosed by a spathe of large leafy bracts, from which emerges a bundle of silky stigmas which may be over six inches long. The pollen grains are heavy and are weighted at one end with large starch grains. They drop directly from the stamens to the stigmas of the same or of adjacent plants.

Section 3. Fertilization

Eschscholtzia californica (Californian Poppy).—Cut off stigmas from several fully open flowers. Mount them dry on a microscope slide and examine uncovered. Select one which shows pollen grains attached, and mount in plain Lacto-phenol or Lacto-phenol in which a stain such as Soluble Blue or Acid Magenta has been dissolved.

The surface of the stigma is formed of short, rounded, projecting cells, like hairs, among which the pollen grains lodge (Fig. 58).

The grains are round and germinate through a **single pore.** The **pollen tubes** can easily be seen, penetrating between the **surface papillae** of the stigma, entering the tissues of the style and then turning downwards towards the base. Frequently the pollen tube has to make a complete turn through 180 degrees to bring itself into the right direction. The stimulus for this movement of orientation is chemical,[1] and is the result of the secretion of Dextrose by the stylar cells.

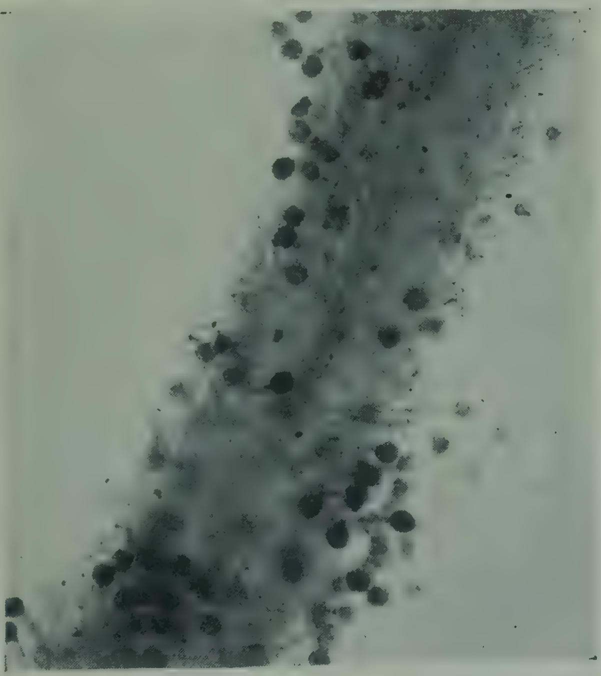

FIG. 58.—*Eschscholtzia californica.* Pollen developing on the stigma.

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[1] See under Chemotropism, Part VII, page 376.

Narcissus majalis.—The pollen is available in the spring months, and it germinates well in 7 per cent. Sucrose solution. It is best to use the common Pheasant's Eye Narcissus (*N. majalis*) or one of the other natural species, as the garden hybrids produce a large percentage of abortive pollen grains.

Mount the fresh pollen in the sugar solution, using either a slide with a hollow depression (cavity slide) or, if that is not available, make a small ring of thin wire, or even sewing cotton, and lay it in the mixture of sugar and pollen on the slide so as to hold up the cover-glass and maintain a shallow space between it and the slide. Cut off a small portion of stigmatic tissue with a razor, place it in the centre of the drop of sugar solution and add a cleaned cover-glass. Place the whole slide in a shallow glass dish or tightly closed box, with a pad of wet filter paper to keep the atmosphere moist and prevent evaporation from the slide.

After a few hours most of the grains will have germinated and pollen tubes will be showing. The stigma tissue obviously diffuses some chemotropic substance, for it is striking to see the way in which the tubes turn towards the centre, where the stigma tissue lies. This action must greatly facilitate fertilization in nature, and it is responsible for leading the pollen tube into the stigmatic tissue and eventually towards the micropyle of the ovule in the ovary.

Each grain only forms one tube. The tube wall is thin and is continuous with the intine of the grain which is pure cellulose. The contents of the grain empty themselves into the developing tube, which is therefore at first densely filled with cytoplasm, enclosing starch grains and the vegetative and generative nuclei.

Later, as the tubes become longer, the empty pollen grains may be cut off from the cytoplasm in the tube by the formation of **callose plugs.**

If a plant of *Echeveria retusa* is available it may be brought into flower by May in a greenhouse, and it provides extremely useful pollen which, under the above treatment, germinates immediately and produces long pollen tubes within an hour. This plant is a common bedding plant, with grey succulent leaves in a rosette and orange-coloured flowers.

Section 4. Embryos and Seeds

A. EMBRYOS

Capsella bursa-pastoris (Shepherd's Purse).—A series of stages in the development of the embryo may be easily obtained in this plant, but for the early stages, at least, sections cut with a microtome are practically essential. If no means of cutting serial sections is available the student should try embedding ovaries for cutting by hand. Ovaries of the appropriate size should be cut open at both ends and fixed in a mixture of glacial Acetic acid one part, absolute Alcohol three parts. Leave them in the fixative for half an hour, then wash out thoroughly in absolute Alcohol for several hours. For further procedure in embedding for section cutting see page 27.

Stage 1.—Ovaries 1·5 mm. long. The ovule is **campylotropous,** that is to say, the **embryo sac** is bent double, the embryo developing at the end of the limb nearest the micropyle. At the **chalazal end** a remarkable development of large (tapetal) cells takes place just below the end of the embryo sac, which are apparently nutritive in function. In this development the **antipodals** also take part. At this early stage the **embryo** consists only of a very large **basal cell,** nearest the micropyle, then a row of four to six small cells, the **suspensor,** and at the top the actual **embryo,** which in the earliest stage consists of one rounded cell. This divides first longitudinally, then twice transversely, at right angles, forming a group of eight cells. This **octant** is an easily recognizable stage which is common to practically all Angiosperms. It may be found in 2 mm. ovaries (Fig. 59).

Stage 2.—Ovaries about 2·0 to 2·5 mm. long. The embryo has now formed a **dermatogen** by a series of **periclinal divisions,** that is, divisions which form new cell walls tangential to the outer surface. This does not occur, however, at the base where the embryo is attached to the suspensor. After the dermatogen cells have been cut off there still remains a group of eight internal cells. Ovaries 3 mm. long will probably show this last stage.

Stage 3.—Ovaries 3·5 to 4·0 mm. long. The eight internal cells undergo repeated divisions, and the central cells of the resulting cell mass become elongated, forming the first rudiments of the **conducting tissues.**

Stage 4.—Ovaries 4·5 to 5·0 mm. long. Two lobes begin to develop at the upper end of the embryo, which eventually become two **cotyledons,** while between them is a single cell which gives rise to the apical meristem of the stem, and forms the **plumule** or first apical bud. At the other end of the embryo a group of cells form the meristem of the **primary root.** The strand of elongated provascular cells lies in an axial line between these two opposite poles of the embryo.

When the cotyledon lobes are about equal in length to the rest of the embryo, division walls begin to appear in the protoplasm of the **endosperm,** forming first round the periphery of the embryo sac. This tissue formation is, however, never completed in *Capsella.* The endosperm degenerates and finally disappears when the embryo is mature.

Stage 5.—Ovaries 5·0 to 7·0 mm. long. After Stage 4 development is very rapid and the embryo soon matures. It bends over and fills the whole of the curved embryo sac, cotyledons and axis thus being doubled over, and lying parallel to one another. Suspensor, antipodals and endosperm all disappear, though the basal cell remains, and a **root cap** is formed. A minute bud develops between the cotyledons and becomes the plumule. The two integuments meanwhile become cuticularized and their cells

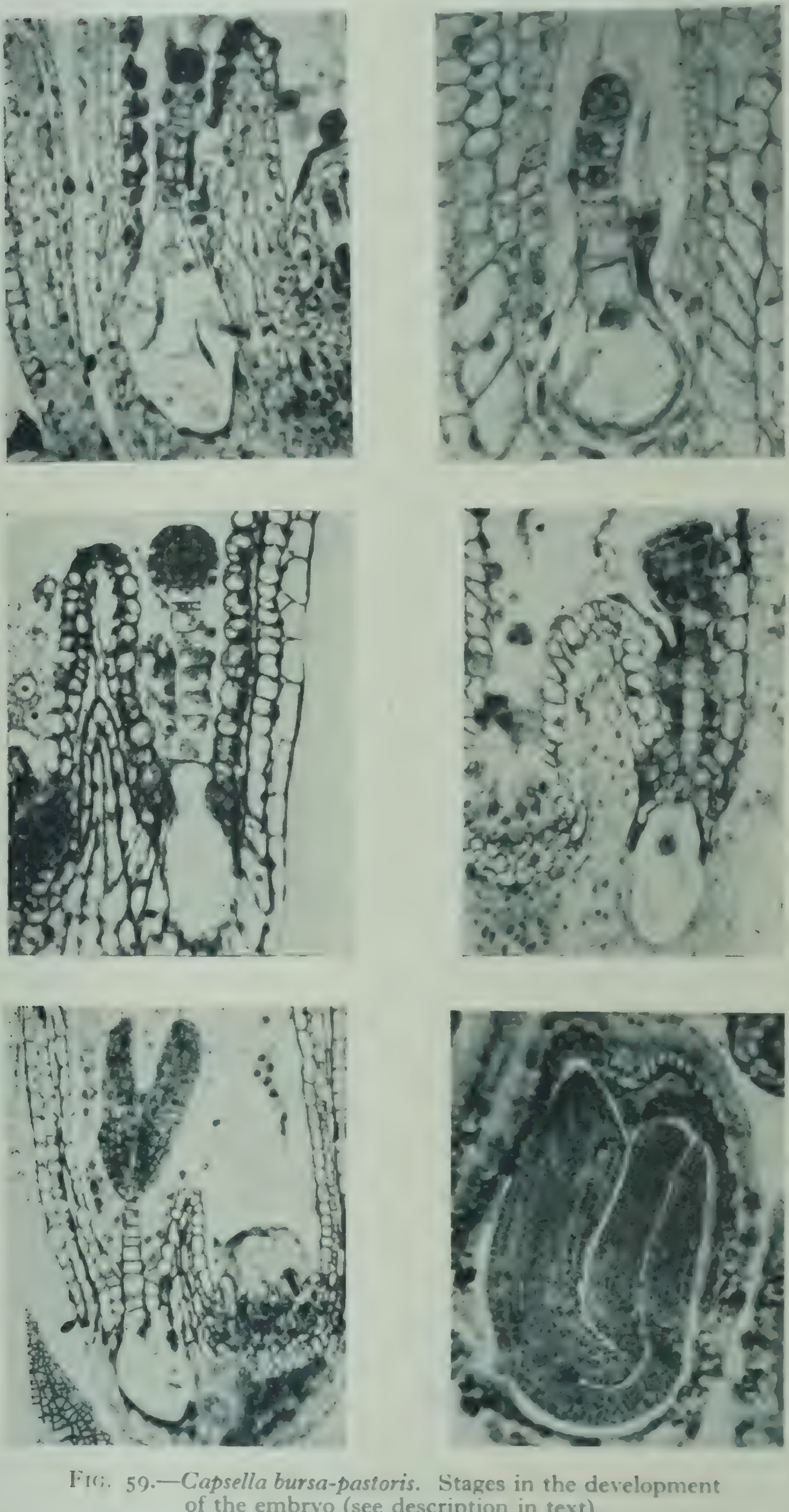

FIG. 59.—*Capsella bursa-pastoris.* Stages in the development of the embryo (see description in text).

thickened to form the **testa** of the **seed** into which the ovule has now developed.

Alisma plantago-aquatica (Water Plantain). — Monocotyledonous embryos are not as a rule so easily examined as those of Dicotyledons. Grass embryos are an exception to this, but they are very specialised in structure. A good generalised type is *Alisma*, which produces uniovulate fruits (achenes) from which the embryos may be squeezed out whole. The achenes are wedge-shaped. The ovule is visible through the flat sides and may be dissected out under a magnifying glass. Mount the ovules in dilute Glycerine and press gently on the cover-slip with a needle, when the embryo sac will burst and the embryo come out whole. Early stages should be looked for before the petals have been shed.

1. Embryos up to the " octant " stage are closely similar to those in *Capsella*. The basal cell is unusually large, at least as large as the remainder of the embryo, and the suspensor is shorter than in *Capsella*, usually only two cells long.

2. After the octant stage the spherical embryo forms a dermatogen layer by periclinal (tangential) cell divisions, and the body enlarges into an oval form, but without any division into two cotyledons. The whole distal end is one cotyledon, and the growing point forms at its base, *i.e.*, about mid-way along one side of the embryo. This growing point remains quite undifferentiated until germination begins.

3. The cotyledon elongates considerably and becomes pointed and slightly curved ; the growing point enlarges to form a minute conical hump of cells, sunk in a slight depression on the side of the embryo. Further than this there is no development until germination, when the cotyledon comes above ground as a green leaf, and the growing point produces the first plumular foliage leaves.

B. SEED STRUCTURE

1. DICOTYLEDONS

Vicia faba (Broad Bean).—The seed swells considerably when soaked for twenty-four hours, and then shows the following external features.

The **testa**, or seed coat, is a greenish-yellow outer skin which is developed from the integuments of the ovule. At one end is the brownish scar of the **hilum,** marking the position of attachment to the **placenta.** At one end of the hilum may be seen a raised triangular patch of testa, marking the position of the **primary root** of the seedling, while between this and the end of the hilum is the **micropyle,** which still exists and is visible in the mature seed. It may be seen with a hand lens as a tiny pore lying exactly at the end of the hilum. The radicle lies in a pouch of the testa, just below the micropyle, and it is here that germination starts.

Remove the testa with a scalpel, beginning at the end farthest from the hilum. There is no endosperm, so that everything inside the testa belongs to the **embryo.** The primary root is under the triangular patch above-mentioned, and just behind the micropyle, where it lies in a pocket between the outer testa and the inner testa or **tegmen.**

Separate the two large **cotyledons,** which are closely sutured to one another along their edges, and between them will be seen the small folded **plumule** with the primary root or **radicle.** If the latter is cut lengthways the early differentiation of the **stele** can easily be seen.

The plumule is really a bud, and from it the whole of the epicotyledonary shoot system develops.

> ***Vicia faba*** (Broad Bean).—Transverse section of the testa of a freshly soaked seed. Sections must be thin. Mount in Glycerine.

The structure of many seed coats is very complex and this is no exception, but the main outlines are clear enough. There is an outer layer of elongated prism-like cells with extremely thick walls which is the main protective layer or **testa.** The walls of these cells are, however, split longitudinally into six or more segments, with narrow channels between. The depth of these channels makes them protective against evaporation of water from within ; but they must cause rapid capillary absorption of water when brought into contact with it outside.

Beneath the prismatic layer is an " arcade " of intercellular spaces, bridged by columnar cells which connect the looser, inner tissue of the **tegmen** to the true outer testa. Through the spaces of this arcade, water absorbed at one spot may readily spread tangentially round the seed.

> ***Phaseolus vulgaris*** (Kidney Bean).—Cut the soaked seed transversely, with a razor, at a point about 3 to 4 mm. from the micropyle towards the nearest end of the seed. Spread Iodine solution with a glass rod over the cut surface.

The two large **cotyledons** will be seen in semicircular section, their flat faces inwards, towards each other. In the space between them are portions of the **plumule,** while at one end of the section is the **radicle,** cut across and lying in a pocket between the cotyledons and the **testa.**

Iodine shows starch in the cotyledons but none in the radicle or plumule.

Split the seed lengthways and the radicle and plumule will be revealed in their position of attachment. Note that the radicle is better developed than the plumule.

> ***Ricinus communis*** (Castor-Oil Plant).—Crack and peel off the hard testa.

Inside is the mass of white **endosperm,** covered by the delicate papery **tegmen** (inner integument of the ovule), with a network of vascular bundles. The area from which this network originates is the **chalazal** end of the seed ; the **embryo** lies at the other end. Cut the seed lengthways in the middle of the flattened sides. The two halves of the endosperm will be seen, with a slit between them, in which lie the two delicate white **cotyledons** face to face. The embryo is very small and lies amidst slightly darker endosperm at one end. If the halves of the endosperm are separated it will be seen that the cotyledons are leaf-like with net venation. The endosperm contains oil and protein but no starch.

The seed bears a small yellowish protuberance at one end which is known as the **caruncle.** It is an outgrowth of the testa.

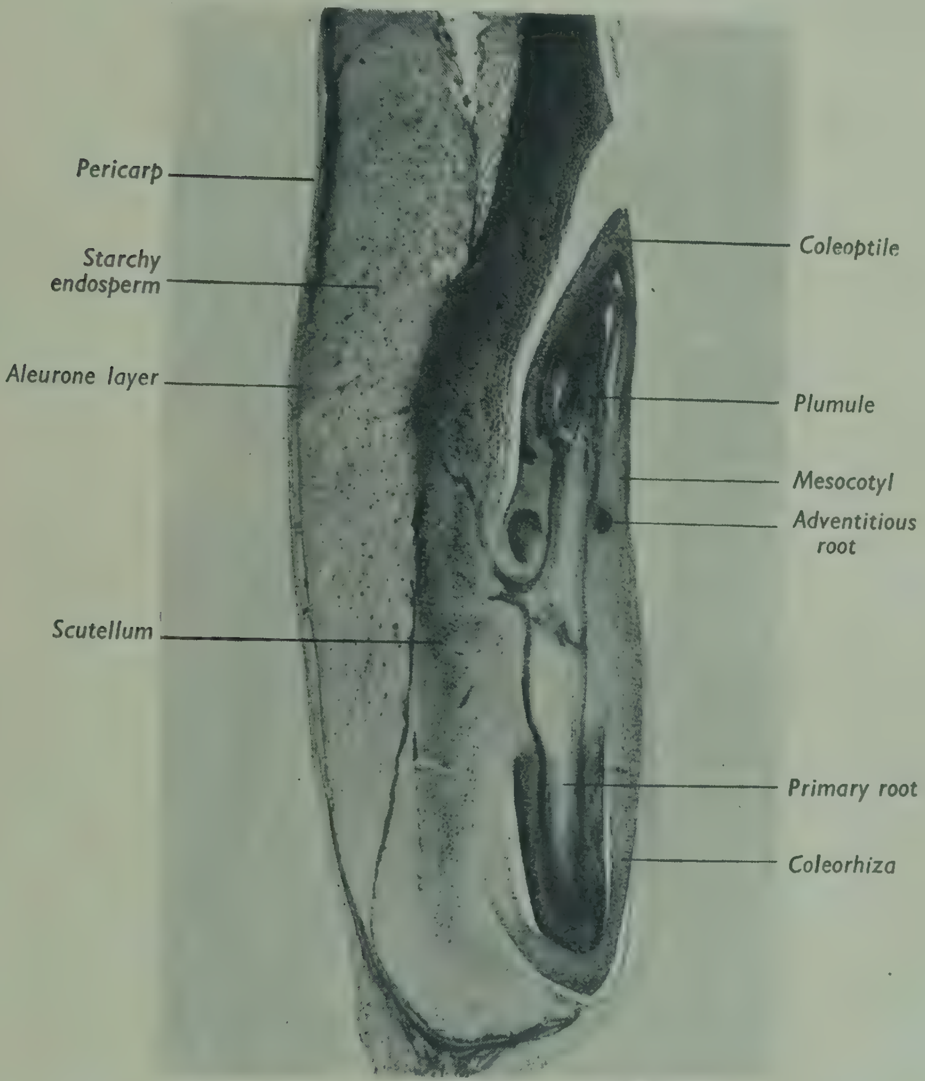
Pericarp
Starchy endosperm
Aleurone layer
Scutellum
Coleoptile
Plumule
Mesocotyl
Adventitious root
Primary root
Coleorhiza

FIG. 60.—*Zea mais.* Longitudinal section through the seed with the embryo.

2. MONOCOTYLEDONS

Zea mais (Maize).—See also *Triticum* (Wheat), page 261. The grain (**caryopsis**) shows a whitish patch on one side which lies over the embryo. Cut the seed lengthways through the middle of this patch.

The contents are divided obliquely into a yellow portion, the **endosperm,** and a white portion, the **embryo.** The part of the latter lying next to the endosperm has the form of a thick shield, the **scutellum,** to which the rest of the embryo is attached laterally (Fig. 60). It consists of the **plumule,** surrounded by the **coleoptile,** and the **primary radicle,** surrounded by the **coleorhiza.** These structures are primary sheaths of plumule and radicle respectively.

The scutellum is considerably larger than the rest of the embryo, but it is, nevertheless, probably the **cotyledon.** The endosperm gives the starch reaction with Iodine, but the embryo does not. The Iodine brings out more clearly the details of the embryo, and shows plainly its attachment to the scutellum.

> ***Allium cepa*** (Onion).—Inspection of the small black seed will show that it has one relatively flat side. Cut a thin slice off this side with a razor so as to reveal the contents of the seed.

The embryo will be seen curled round like a little white maggot in the translucent **endosperm.** One end of the embryo lies close to the seed coat. This is the **radicle.** The other (inner) end is a tubular **cotyledon,** inside which is the **plumule,** which cannot be seen until after germination. There is no starch in the endosperm.

Section 5. Germination

A. DICOTYLEDONS

1. Non-Endospermic, Hypogeal.

Vicia faba (Broad Bean).—The primary root emerges at or beside the **micropyle,** below which it lies, and turns directly downwards. As it elongates it raises a triangular flap of the testa, being aided therein by the swelling of the two shoulders of the cotyledons. The base of the root then affords a *point d'appui* or fulcrum by means of which the young plumular shoot (**epicotyl**) withdraws itself from between the cotyledons. The latter are not withdrawn, but remain inside the testa, thus being **hypogeal.** The cotyledons are large and fleshy, and fill the whole space inside the testa. They contain food reserves (starch, etc.), and thus physiologically replace the missing endosperm.

When the epicotyl has grown an inch or two in length the axillary buds of the cotyledons may be seen beginning to develop and emerge at its base. The root meanwhile is producing branches, and becomes corky at its base.

2. Non-Endospermic, Epigeal.

Phaseolus vulgaris (Kidney Bean).—The seed is, like the Broad Bean, non-endospermic, with large fleshy cotyledons, but in this case the **hypocotyl** elongates by intercalary growth and emerges in the form of a crook, or elbow, which rises to the surface of the soil bringing the cotyledons with it. The crook does not straighten out until the light is reached, when the cotyledons separate, and the **plumule** and **epicotyl** grow out from between them. The cotyledons turn green and apparently function as leaves.

3. Endospermic, Epigeal.

Ricinus communis (Castor-Oil Plant).—As the root emerges the hard testa cracks like a nutshell revealing the white **endosperm.** The **cotyledons** are embedded face to face in the middle of this endosperm, and for some time, while the root system develops, they remain embedded, absorbing food from the endosperm until it is reduced to little more than a membrane surrounding them. Meanwhile the **hypocotyl** has been elongating so that testa, endosperm and cotyledons are all raised into the air, the top of the hypocotyl being hooked, so that the cotyledons bend downwards. When the endosperm is exhausted the hypocotyl straightens out and the cotyledons emerge from the remains of the endosperm and turn green, enlarging at the same time until they appear as ovate leaves 1 to 2 in. across.

B. MONOCOTYLEDONS

1. Non-Endospermic, Epigeal.

> **Orchis,** or any other available type of Orchid. The seeds of Orchids are extremely small and are not easy to germinate, as they require to be infected with their specific mycorrhizal fungus. One good way is to use soil from the same pot in which the parent plant is growing. Fill a small pot with it, and place on the soil a piece of damp linen the edges of which are covered by the soil and, if you like, some moss. Sow the seeds on the linen and keep the whole in a very moist place.

The embryo before germination is quite undifferentiated, and at first it simply enlarges into a tiny turnip-shaped mass of cells, 1 to 2 mm. across, the **primary tuber,** which becomes green. Then a growing point is formed at the broad end, and from this one green cotyledon appears, followed by other leaves in alternate succession.

Unicellular rhizoids are formed on the primary tuber but adventitious roots do not appear for some time.

Triglochin and *Aponogeton* are the only types which may be available to illustrate the **non-endospermic, monocotyledonous** seed with **hypogeal** germination. The first genus has two species in the British flora, *Triglochin maritimum* and *T. palustre* ; the second genus is frequently grown, especially *Aponogeton distachyon*, as a pond plant in gardens, but neither is easily obtained as seed for germination purposes. We have therefore simply named them as examples of this rare type without detailed description.

2. Endospermic, Hypogeal.

Triticum vulgare (Wheat).—See also *Zea mais* (Maize), page 259. The grain is really a one-seeded fruit (**caryopsis**) in which testa and pericarp cohere to form one covering membrane. The greater part of the interior is filled with **endosperm,** and the **embryo** can be seen, as a minute yellowish patch, at one end of the convex side of the grain. The **cotyledon** is usually considered to be represented by the **scutellum,** which lies between the endosperm and the embryo, and which remains embedded in the grain during germination. The epicotyledonary **plumule** grows straight upwards on emergence, its first leaf, the **coleoptile,** forming a whitish sheath around it, from the apex of which the later leaves emerge.

The primary root aborts at an early stage in the formation of the embryo, and its place is taken by three **adventitious roots,** which emerge each surrounded by a sheath, the **coleorhiza,** which may be seen as a collar round the base.

3. Endospermic, Epigeal.

Allium cepa (Onion).—The **radicle** emerges first and grows downwards. The **tubular cotyledon** is gradually withdrawn from the **endosperm** by basal growth, coming above ground in the form of a crook, one end in the seed and the other attached to the radicle. The cotyledon turns green and continues growth in a sharply bent form until about 1 in. high. Then the tip either leaves the seed or lifts the seed with it from the soil, and the cotyledon partially unbends, so that its two halves are more or less at right angles. At this stage a slit forms at the base near the radicle, and from this the first leaf of the **plumule** emerges, followed in turn by others, so that the growing point comes out into the open and supplants the cotyledon, which thereafter withers away.

Section 6. Fruits

A. DRY FRUITS

1. INDEHISCENT

(*a*) Akene.

Ranunculus acris (Buttercup).—The fruits are minute and one-seeded and do not open to set free the enclosed seed. Such fruits are called **akenes** or **achenes,** and in Buttercup the group of akenes clustered together in fruit is called an **eterio of akenes.**

Akenes are characteristic of many members of the Buttercup family, *e.g., Clematis, Thalictrum, Anemone.*

(*b*) Caryopsis.

Triticum vulgare (Wheat) or ***Zea mais*** (Maize).—The grasses have a special form of akene, the **caryopsis** or grain, in which the testa is completely and indissolubly united to the **pericarp** or fruit coat. Fruit and seed thus become a single structure which very closely resembles a true seed.

(*c*) Cypsela.

Taraxacum officinale (Dandelion).—Most of the Compositae possess an akenal fruit formed from an inferior ovary, which develops at its apical end an outgrowth termed the **pappus,** a feathery umbrella-like structure which assists in wind distribution, and is homologous with a calyx. After the fruit has reached the ground, hygroscopic movement of the pappus may draw the fruit along the surface of the soil till it lodges in a cavity, thereby assisting in burying the seed.

(*d*) Samara.

Ulmus procera (Hedgerow Elm).—The fruit is a **winged akene,** in which the wing is developed around the margin of the fruit, and is derived from the **pericarp.** The extent of the wing varies, but when well developed may assist in the wind distribution of the fruit.

Fraxinus excelsior (Ash).—In this type the fruit is also an akene but the wing is longer and is formed by an elongation of part of the pericarp into a long blade-like outgrowth termed the " key." The wing assists in the wind distribution of the fruit.

2. DEHISCENT

(*a*) Follicle.

Aconitum napellus (Monkshood) or ***Delphinium*** or ***Aquilegia.*** Each carpel produces a separate fruit with many seeds, which is like a small legume or pea-pod, except that it opens down one side only, to release the seeds. The **pericarp** is papery.

In the examples cited above the fruits are grouped into an **eterio of follicles.**

(*b*) Legume.

Pisum sativum (Pea) or ***Sarothamnus scoparius*** (Broom).—A type of fruit which gives its name to the great order of Leguminosae, which, with few exceptions, produce this type of fruit in some form. It is the product of a single carpel and has seeds arranged down one side only, but it splits by two lines of dehiscence into two **valves.** In certain types, *e.g.*, *Cytisus*, this dehiscence takes place explosively, the two valves then curling spirally like springs and throwing off the attached seeds.

(*c*) Capsule.

Oenothera biennis or other species.—The **capsule** is a hollow dehiscent fruit composed of several carpels (in this case four) which split open or split apart to release the seeds. In *Oenothera* the line of dehiscence, or splitting, runs down the middle of each **loculus** of the fruit, which is therefore called **loculicidal.** At the same time the **septa** spring apart at the **axis** so that the whole pericarp separates into four segments.

Scrophularia nodosa (Figwort).—This is a common plant of moist places, 2 to 3 ft. tall and with small brown flowers. The capsule consists of two carpels only, which dehisce down the septum, which is thus split into two halves lengthways. This is called **septicidal** dehiscence. It is also well shown by species of *Hypericum*.

Melandrium dioicum (Rose Campion).—The capsule is formed of five carpels so completely united that the interior is **unilocular** with no dividing septa. From the base of this loculus rises the **placenta,** like a column, with the seeds attached all over it. It is described as both **free** and **central,** as it is not attached to the carpels at any point.

The capsule opens by the folding back of five flaps or **valves** at the top, representing the apices of the five carpels. This is called **valvate** dehiscence.

Such capsules are common in this family, the Caryophyllaceae.

Papaver dubium or other species.—The Poppy fruit is a capsule of many united carpels, divided by **incomplete septa,** which do not reach to the centre of the fruit. When ripe the outer pericarp wall shrinks lengthwise and separates at the top from the stigmatic cap of the fruit. This leaves a ring-like opening all round which is divided into compartments by the exposed edges of the septa.

This is called **porose dehiscence.** Seeds can only escape if the fruit is violently shaken, when there is a chance that they may be flung to a distance. Such a method of facilitating dispersal of seeds is sometimes called the **censer mechanism.**

Anagallis arvensis (Scarlet Pimpernel).—The minute capsules are formed of five carpels with no septa between them, so that the interior is **unilocular.** Dehiscence is by a circular split round the equator of the capsule, **circumscissile,** the top coming off like a lid. This special form of capsule has the name **pyxidium.**

(*d*) Siliqua.

Matthiola incana (Garden Stock).—The great family of the Cruciferae, of which it is an example, produce a special type of capsule fruit called a **siliqua,** which is formed of two carpels, usually long and narrow, partially separated internally by two **incomplete septa** which overlap. There are two rows of seeds in each carpel. When ripe the carpel walls start to separate from the base upwards and leave the septa, framed in a thickened rim, forming the **replum** of the fruit. The seeds remain attached to the rim of the replum and are gradually shaken off. Many are flat or winged, which facilitates wind-dispersal.

When a siliqua is short and broad, as in *Capsella* (Shepherd's Purse), it is sometimes called a **silicula.**

3. SCHIZOCARPIC

Schizocarp.

Heracleum sphondylium (Hog Weed).—This common umbellifer shows very well a type of fruit, common in its family, in which the ripe fruit divides into portions, each of which when separated is an **akene.** Originally two one-seeded flattened carpels are joined to form a bilocular capsule, but they separate at maturity along their flat faces. The split runs from below upwards, and the two halves remain for some time attached by little bridges to a central axis called the **carpophore.**

Althaea rosea (Hollyhock).—The fruit is somewhat similar to that of *Heracleum*, except that there are many one-seeded portions which are arranged in a ring round the base of the united styles. The parts separate in the form of **akenes** when ripe.

B. FLESHY FRUITS

1. BERRY

Ribes uva-crispa (Gooseberry).—This fruit is a typical berry in everything except that it is formed from an inferior ovary and is hence, strictly speaking, a pseudocarp. The outside of the fruit is the succulent **pericarp,** with an outer skin of receptacle tissue completely united to it. There are two **placentae,** and the seeds therefore form two groups. The testa of each seed has a succulent outer coat, all of which together form the inner pulp.

The **endocarp** layer of the pericarp is a delicate membrane round the cavity in which the seeds lie.

Lycopersicum esculentum (Tomato).—The **pericarp** is soft throughout, and the interior is divided (normally) into two **loculi** by a **septum** which bears two highly developed **placentae.** On these are borne the numerous seeds, each with a pulpy outer testa, which, as in the Gooseberry, make up the inner pulp of the fruit.

Vaccinium macrocarpum (Cranberry).—This is similar to the above, except that the seeds lack the pulpy testa.

2. DRUPE

Prunus cerasus (Cherry).—The **pericarp** is differentiated into a soft part, **epicarp,** or skin, and **mesocarp,** or flesh, and a hard part, **endocarp,** which is stony and encloses the seed. The latter is commonly known as the " kernel " and possesses only a thin papery testa, as it is sufficiently protected by enclosure in the stony endocarp.

Drupaceous fruits are all essentially similar. Other familiar examples are the Plum, Apricot and Peach. The Raspberry and Blackberry are aggregates of small single fruits, each of which is a miniature drupe or **drupel.** The Coconut, when in the complete state, is a drupe with a fibrous mesocarp. The portion sold in shops is only the endocarp containing an endospermic seed.

C. PSEUDOCARPS

Fragaria vesca (Wild Strawberry).—The fleshy portion is formed from the receptacle of the flower which becomes much enlarged and coloured. On it are the very numerous minute carpels or "pips," each of which is a separate **akene**; these are the true fruits of the plant. Many of the akenes still retain their **styles** and **stigmas.**

At the base is the persistent **calyx,** with five supernumerary members, which form the **epicalyx.**

Pyrus malus (Apple).—The fruit is formed from an inferior ovary and is therefore enclosed in the tissue of the receptacle which forms all, or at least the greater part, of the flesh. At the opposite end from the stalk is the remains of the **calyx** surrounding a small hollow, at the centre of which may be some remnants of the **stigmas.** When cut open lengthways the true **carpels** are seen as hollow spaces, usually five, in the centre of the flesh, surrounded by horny walls and containing the **seeds.** It is probable that the innermost flesh, in which there is an independent set of vascular bundles, is really part of the carpel walls, but there is no discontinuity between it and the outer flesh, which represents the receptacle tissue.

Ananas sativa (Pineapple).—This is not a single fruit but an entire inflorescence in the form of a spike, the flesh being composed chiefly of the axis, the fleshy bracts and the fleshy calyces all closely united into one mass. The tips of the bracts are free and appear as scales on the outer surface, partially covering the remains of the flowers. Usually no seeds are formed, and the plant is propagated vegetatively. The inflorescence is not terminal, and the axis continues its development to form a group of leaves above it. This portion may be cut off and rooted separately.

Ficus carica (Fig).—The axis of the inflorescence is hollow, with the flowers enclosed inside, only a small aperture being left at the top through which small insects enter and effect pollination. The female flowers occupy the lower portion, the male flowers are formed only near the top.

Section 7. Dispersal Mechanisms

A. MECHANICAL DISPERSAL

Viola odorata (Sweet Violet).—The capsule is triangular in section and is formed of three carpels, but is unilocular. At maturity the carpels split apart and spread out starwise. Each is V-shaped in section and contains two rows of seeds attached marginally. As the carpel dries, it contracts, and the seeds are tightly compressed between the edges of the V. The seeds have hard glossy coats, and when a pressure is reached sufficient to tear them loose from their funicles, they are shot out violently in the same way that a cherry stone may be shot from between the fingers by pressure.

Geranium robertianum (Herb Robert) or other species.—The flower has five carpels, each producing a single seed, and with five long styles joined together to form a central column in the flower. When ripe, each carpel splits away from the others, progressively from below upwards, the style rolling up as it does so. At the same time the carpel wall splits open below and the separation, splitting and rolling up, is carried out with such rapidity that the seed is shot out as by the action of an underhand bowler.

The styles remain attached to the central column at their tips, the five empty carpels dangling round about from their springy supports.

This schizocarpic type of fruit in which the carpels split apart and release the contained seeds is called a **regma.**

B. WIND DISPERSAL

1. SEEDS

Epilobium montanum, or other species (Willow Herb).—This plant gets its English name from the resemblance of its plumed seeds to those of *Salix*, the Willow, which may be used as an alternative type. The fruit is a capsule which dehisces to release large numbers of minute seeds, each with a plume of hairs at one end, by means of which they are readily carried away on the wind.

This plume is morphologically an **aril,** that is, an outgrowth from the seed **testa.**

Chelone barbata (a well-known garden plant).—Each seed is about 2 mm. long, light and flat, and surrounded by a membranous wing which catches the wind. This wing is, once more, an **aril.** Seeds of *Chelone* can normally be bought, but if it is not available the following, among many others, also produce winged seeds : *Lunaria* (Honesty), *Catalpa*, *Eccremocarpus* and *Lilium.*

2. FRUITS

Clematis vitalba (Travellers' Joy).—Each fruit is an **akene** and is surmounted by the persistent **style** which elongates to about an inch in length. Both akene and style produce many fine hairs which make an effective plume to catch the wind.

Tragopogon pratensis (Goat's Beard).—Each fruit is a **cypsela,** *i.e.*, an akene produced from an inferior ovary. The apex of the gynoecium elongates, after the fall of the other floral parts, into a long tube, bearing at its upper end an umbrella of radiating fibres, the **pappus,** which represents the calyx. These fibres bear fine lateral hairs which interlace, the whole forming a very effective parachute. The akenes are covered with small bristles, directed forwards, which catch and anchor the fruit as it drags along the ground after falling from the air. *T. porrifolius* (Salsify) is similar but larger. Many other Compositae have similar plumed akenes, *e.g.*, Dandelion and Thistle, or, without the pappus tube, *Senecio vulgaris* (Groundsel).

Betula pendula (Birch).—The akenes have a brownish membranous wing forming a heart-shaped border. The structure is a fruit (**samara**) and the wing, therefore, is an outgrowth of the pericarp, but otherwise it closely resembles the winged seed of *Chelone* mentioned above.

Ulmus procera (Hedgerow Elm).—This produces similar but larger winged akenes. They are, however, normally sterile.

Fraxinus excelsior (Ash).—This has akenes with a wing produced at one end only.

Carpinus betulus (Hornbeam).—This produces akenes to which the three-lobed bract from beneath the flower remains attached. This broad bract acts as a sail in the same way as the wing of a samara.

Humulus lupulus (Hop) has a **strobilus** or cone of bracts, each bearing an akene fruit in its axil, the product of one flower. The whole strobilus behaves as a unit for dispersal. The dry papery bracts act as wings to assist the process.

C. ANIMAL DISPERSAL

Bidens cernua (Bur-Marigold).—A common plant of pond margins and ditches with an akene (**cypsela**) about 1 cm. long, crowned with a pappus which consists of two to four sharp teeth (3 mm.) each of which is furnished with reflexed bristles. These catch easily in fur or clothing, and cause the fruit to be carried about and dispersed by animals.

Arctium minus or other species (Burdock).—The involucral bracts are each furnished with a sharp lignified terminal hook which readily catches in any rough material. This causes the whole capitulum to be pulled off the plant and carried away.

Galium aparine (Cleavers) and ***Myosotis sp.*** (Forget-me-not).—These also have very effective hooked hairs on the pericarp.

Geum urbanum (Herb Bennet) has hooks formed of the stigmas which become spiny after fertilization.

D. WATER DISPERSAL

Cocos nucifera (Coconut).—If a specimen of the complete fruit is obtained it will be seen to be a **drupe,** of which the shell is the stony endocarp, surrounded by a thick fibrous mesocarp and a leathery epicarp.

In this condition the fruit is very buoyant and will remain floating, especially in sea water, for many weeks. As the natural habitat of the Coconut is on or close to tropical seashores, this capacity for flotation has enabled the ocean currents to disperse the nut far and wide, so that its distribution is now pan-tropical.

Caltha palustris (Kingcup) is dispersed by flotation. Each seed has a light spongy aril at one end, which acts as a float ; it is best seen if the seed be cut open lengthways.

Oenanthe crocata (Water Hemlock) has a schizocarp formed of two akenes. Each of these has two corky ribs, one at each edge, which appear to be floats. The plant is a waterside weed and is dispersed by flotation.

PART SIX

CLASSIFICATION OF ANGIOSPERMS

A Selection from the International Rules of Botanical Nomenclature (1910)

ARTICLE 1.—Natural history can make no progress without a regular system of nomenclature, which is recognized and used by the great majority of naturalists in all countries.

ARTICLE 7.—Scientific names are in Latin for all groups.

ARTICLE 10.—Every individual plant belongs to a *species*, every species to a *genus*, every genus to a *family*, every family to an *order*, every order to a *class* and every class to a *division*.

ARTICLE 15.—Each natural group of plants can bear in science only one valid designation, namely the oldest.

ARTICLE 19.—Botanical nomenclature begins for the different groups of plants at the following dates: *e.g.*, Phanerogamae and Pteridophyta, 1753 (Linnaeus, *Species Plantarum*. Ed. 1).

ARTICLE 24.—Genera receive names, substantives or adjectives used as substantives, in the singular number and written with a capital letter, which may be compared with our own family names. These names may be taken from any source whatever and may even be composed in an absolutely arbitrary manner.

ARTICLE 26.—All species, even those that singly constitute a genus, are designated by the name of the genus to which they belong, followed by a name termed *specific*, usually of the nature of an adjective forming a combination of two names, a binomial or binary name.

Section 1. The Description of the Flower

A. FLORAL DIAGRAMS

A floral diagram is an ideal ground plan of a flower by means of which many of the characteristics of its parts and symmetry can be expressed in graphic form. There is a certain art in the construction of a good floral diagram which is only learnt by practice, and the beginner would do well to copy, on an enlarged scale, some of the diagrams to be found in every book on Systematic Botany, comparing them, whenever possible, with the original flowers. It is better to learn by copying in this way than to start floundering for oneself with only a few rules for guides, in what is admittedly not always easy.

Nevertheless it helps to know the rules from the start.

Firstly, the diagram is conventionally always circular, departures from symmetry in the flower being marked by differences in the size and form of the individual parts in the diagram. The whorls of parts are represented on concentric circles, sepals on the outside, then petals, stamens and carpels in order. In the rare cases of spiral flowers the spiral arrangement must be drawn carefully inwards to the centre.

Start with a circle not less than 2 in. diameter for the sepals, or the carpels will be unduly crowded.

Secondly, the parts should be represented by sections, actual or conventional, drawn in upon the respective circles at positions corresponding to their actual positions. A conventional section is represented by two lines forming a narrow crescent like a paring of finger-nail. To determine the positions, the relationship of the flower to the inflorescence must be observed. Above the diagram draw a small circle with cross lines in it to represent the axis, and if there is a bract draw a conventional section of it below the diagram. The flower is thus shown between bract and axis, which is the true morphological position. Now observe whether a sepal or a space between two sepals stands at the back of the flower. Begin with this sepal and mark in the others to correspond with the number, relative size and position of the actual sepals. Where there is an odd number of sepals the odd member will be either *anterior* or *posterior* to the flower, *i.e.*, opposite the bract or opposite the axis. Do the same thing with the petals, remembering that in most cases the petals *alternate* with the sepals. If the flower is zygomorphic, the petals and perhaps also the sepals will not all be of equal size. This must be attended to in making each part of the diagram. If any portion of the perianth has a spur this may be shown by drawing a little loop at the back of the organ.

Thirdly, if parts of the same whorl are joined together, draw lines to link their edges together, and if parts of different whorls are joined, *e.g.*, stamens to petals, draw linking lines between the parts concerned.

Stamens, one or more whorls, are marked by transverse sections of the anthers, and the gynoecium by a transverse section of the carpel or the ovary.

Where the parts are spirally arranged, care should be taken to count the number in one complete turn of the spiral and mark them on the diagram at corresponding intervals.

In this way the number, form and relationships of the parts of the flower can be shown so far as is possible horizontally.

B. LONGITUDINAL SECTIONS

The features which cannot be shown in the floral diagram can best be illustrated by drawing an accurate, median, longitudinal section of the flower, cut with a sharp razor, from below upwards, and in the case of a zygomorphic flower, along the plane of symmetry. This section may be drawn as a true plane, that is, with outlines showing only what lies actually in the plane of the section, but it is better to treat it pictorially, if you can, to show something of the general appearance of the flower. Such longitudinal pictures can be made very descriptive and valuable if well drawn, but the relationship of the parts must be carefully represented, and all heavy shading or pencil scribble avoided if they are to be of scientific value. If you have a tendency to make dashing sketches, restrain it, and for this occasion confine yourself to the sober levels of representational art.

C. FLORAL FORMULAE

Lastly, there is a handy method of summarizing the characters of a flower by a kind of shorthand notation which gives the essential facts. The various groups of organs are represented by initials, K = Calyx, C = Corolla, A = Androecium and G = Gynoecium. Each letter in turn is followed by the number of parts in its particular group, *e.g.*, K5 means five sepals in the calyx. Brackets round the figures mean that the parts are joined together, thus : C(4). If more than one whorl is present each is shown separately, thus : A5 + 5 + 5. Finally, if the flower is epigynous a line is placed above the G number, if hypogynous it is placed below it, or if perigynous at the side of it. If a group of organs is missing do not ignore it, but put 0, *e.g.*, K0. If the number of parts of a particular group is large and indefinite the sign ∞ is used instead of a number. Thus the formula for a Buttercup is : K5, C5, A∞, G∞, and for a Lily is : P(3 + 3), A3 + 3, G(3). P is used in this case for perianth instead of K and C, as all the parts are alike, and this is a useful variation, though not always adopted by authors.

Two other symbols are commonly added to the Floral Formula, placed at the beginning or the end, namely ⊕ signifying actinomorphic and ·|· signifying zygomorphic. These have been used in the following pages.

Section 2. Examples of the Principal Families

DICOTYLEDONS

A. RANUNCULACEAE

1. ***Ranunculus acris*** or other species. April-September. This family is almost wholly herbaceous and belongs to the mild temperate climate of the northern hemisphere, with very few members either in the Tropics or south of the Equator. The flowers are regarded as "primitive," that is to say, that they show no high degree of specialization in structure, they are nearly all regularly symmetrical, and their parts are indefinite in number and mostly arranged in a spiral succession.

For the description of *Ranunculus* flower, see page 238.

Floral formula : K5, C5, A∞, G∞, ⊕

These flowers are visited by a great variety of flying insects, including bees, but mostly by the Syrphidae (the Hover Flies). The stamens dehisce outwards and at first stand upright in a hedge round the carpels. Visitors alight on the flat petals and thrust down between petals and stamens to reach the small nectar pocket at the base of each petal. In so doing they dust their backs with pollen from the open anthers. At the end of this " male " phase the stamens are shed, thus leaving the carpels exposed. These next bend outwards, so that they occupy the previous position of the anthers and thus collect any pollen from visitors arriving from younger flowers. The " female " phase lasts until pollination is completed and not till then are the petals dropped. The carpels ripen into akenes.

Cross-pollination thus depends fundamentally on the principal of **dichogamy** or separation in time of the ripening of the organs of the two sexes. In this case the flower is **protandrous,** the androecium ripening first, while the opposite case of **protogyny** is found in *Plantago* (p. 252).

2. ***Caltha palustris*** (Kingcup).—March - May. A common plant of marshes and stream sides, whose golden flowers are conspicuous in the spring months. The flower is typically ranunculacean in its open structure and multiplicity of parts. There are no petals, the golden sepals taking their place. The carpels form follicle fruits.

Floral formula : $K5,\ C0,\ A\infty,\ G\underline{\infty},\ \oplus$

For description of the flower, see page 238.

3. ***Aconitum napellus*** (Monkshood).—July - September. This genus is somewhat exceptional in its family in having a zygomorphic flower, but the zygomorphy affects only the perianth, the **stamens** and **carpels** being spirally and regularly arranged. The **sepals** are petaloid, blue and unequal, the posterior one larger than the others and forming a hood over the flower. The two lateral sepals are larger than the two anterior ones. The petals are suppressed or present only as rudiments, except for two at the back which are modified into nectaries and are borne on stalks under the hooded sepal. The type is a useful contrast to the regular flowers of other Ranunculaceae and shows the principle of zygomorphy in a simple form.

Floral formula : $K5,\ C2,\ A\infty,\ G\underline{3\text{-}5},\ \cdot|\cdot$

B. PAPAVERACEAE

Papaver rhoeas (Corn Poppy).—The Poppy family is a small group practically confined to north temperate regions and almost entirely herbaceous. They are notable for their production of **latex,** which sometimes contains important alkaloids such as Morphine in the Opium Poppy (*P. somniferum*), but never produces any valuable amount of rubber. The red Corn Poppy is most at home on light and especially on chalky soils.

Floral formula : $K2,\ C4,\ A\infty,\ G(\underline{\infty}),\ \oplus$

The two green **sepals** are caducous, that is, they fall off very soon. The **petals** are really set in two pairs, *i.e.*, 2 + 2, widely overlapping and of delicate texture. In the bud they are crumpled in a peculiar and characteristic way, and this crumpled character clings to them throughout the flower's existence.

There is no nectar formed, but bees come readily to collect the abundant pollen from the numerous dark-coloured **stamens.** The **carpels** are also numerous and united into a flat-topped gynoecium, which has no styles, but has numerous stigmatic rays on the flat upper surface. Each stigma lies immediately over a division wall in the ovary, but these division walls are incomplete and do not meet at the centre, so that the ovary is really unilocular. Each ovarian septum is thickly covered with minute ovules, and an immense number of tiny seeds are formed. When the ovary is ripe the outer wall shrinks downwards, breaking away from the stigmatic cap and leaving an annular gap round the ovary in which the outer edges of the septa may be seen. The ripe seeds are detached and are shaken out through this open gap, though only when the capsule is swayed by a strong wind. In this way it is ensured that the seeds are flung to some distance from the parent plant.

C. CRUCIFERAE

Cheiranthus cheiri (Wallflower). —May - June. The Cruciferae are mostly herbaceous or shrubby and include several important crop plants such as the Cabbage and Turnip. It is a large family, but its members have a very uniform and easily recognizable floral structure, the most conspicuous features being the four petals, arranged crosswise, and the **siliqua fruit.**

Wallflower is not truly native to Britain, but the yellow flowers of the wild type are a familiar feature of old walls in most parts of the country. Its true home is Central Europe.

Floral formula : $K2+2$, $C4$, $A2+4$, $G(2)$, ⊕

There are no bracts or bracteoles except for the lowermost flowers.

The **calyx** consists of two pairs of sepals, the outer pair being in the median (antero-posterior) line of the flower, and each sepal is usually provided with a honey pouch at the base. The **corolla** has four petals, each divided into a vertical narrow portion called the " claw " and a broad, horizontally expanded portion, the " limb." The claws are set so close together, edge to edge, that without being actually united they form, in effect, a floral tube which restricts the movements of insect visitors just as efficiently as a real tube. The **androecium** is made up of two short lateral stamens and four longer, median stamens, which often arise from two rudiments only, indicating that the basic number of stamens is really four. The **gynoecium** consists of two carpels, joined, between which there is a false septum formed of two incomplete, overlapping septa. The carpels are long and narrow and develop into a cylindrical capsule of the special type called a **siliqua,** in which the outer walls of the two carpels separate at maturity, from the base upwards, leaving the flattened seeds attached to the thickened edges of the false septum. This framed and persistent false septum is called the **replum,** and it is very characteristic of cruciferous fruits.

D. VIOLACEAE

Viola canina (Dog Violet).—April - August. The family is a small one, the genus *Viola* itself containing about two-thirds of the species. They are temperate and mostly montane in their distribution. Small curiously isolated groups of species occur in certain spots, chiefly mountains or islands, in various parts of the world, which suggests a decline from an earlier world-wide dispersal. The flower of *Viola* has already been described on page 239, but we may add here that there are five **sepals,** each with a backward extension beyond its point of insertion on the receptacle, that the lowermost largest **petal** is extended backwards into a long pouch or **spur** and that the **anthers** are flattened, each with an apical appendage, which together fit closely round the style in a compact group. The lowest pair of stamens have also long backwardly directed appendages which lie in the petal spur and act as **nectaries,** the honey being stored in the spur. Lastly, the **ovary** consists of three carpels closely united, so that there is only one **loculus** with three distinct rows of ovules. The stigma is knob-like and produces a flap or scoop which operates in pollination.

Floral formula : $K5, C5, A5, G(\underline{3})$, ·|·

The fruit is a capsule. For seed dispersal mechanism, see Part V, page 266.

The flowers are largely visited by bees, which usually hang head downwards on the flower to insert the proboscis below the stigma into the spur. In so doing the proboscis presses up the two posterior processes of the lower stamens which lie in the spur, and this, by a lever action, lowers these anthers from their position in the group of anthers round the style. The pollen of all five anthers is shed into the space enclosed within the group round the style, and when the bottom pair of anthers are lowered, this pollen falls out on to the base of the bee's proboscis. The withdrawal of the proboscis presses the stigmatic flap forwards and upwards, thus preventing any pollen from reaching it, but when the proboscis is inserted into another flower, this flap acts as a scoop, taking up the pollen which is already there.

In the small field Pansy, *Viola arvensis,* on the other hand, there is no stigmatic flap, and the receptive hollow on the stigma is directed inwards, so that the flower is regularly self-pollinated.

Many species of Violet produce, throughout the summer, flower buds which do not open but are self-pollinated in the bud. These are called **cleistogamic** flowers, and they continue to form perfectly good seed capsules long after the coloured flowers have ceased to appear.

E. CARYOPHYLLACEAE

Stellaria holostea (Stitchwort).—April - June. The family is one of herbaceous plants, both temperate and tropical in distribution and commonly recognizable by their decussate leaves and their dichasial inflorescences with white or red flowers. The parts of the flower are normally in fives, except for a frequent reduction to two or three in the gynoecium.

Stitchwort has large white flowers that are conspicuous in every hedgerow in early summer.

Floral formula : K5, C5, A5 + 5, G($\underline{3}$), ⊕

The **calyx** is formed of pale green, free sepals, and the **corolla** of five petals, free and regularly symmetrical. Each petal is so deeply bifid as to give the appearance, at the first glance, of being double. The **androecium** has one whorl of five longer outer stamens, and one whorl of five shorter inner ones. The former have each a tiny yellow **nectary** outside the base of the filament. Each nectary has a little central pit in which the nectar gathers. There are three **carpels** and three styles, but the ovary is unilocular and the placenta free and central.

There are three stages in the flower development. Firstly, the outer stamens, and secondly, the inner stamens stand close together in the centre of the flower immediately over the stigmas, but with their pollen-shedding surfaces upwards. Lastly, the stamens spread out, making way for the three stigmas which grow above the stamens and spread out, exposing their receptive surfaces to catch pollen brought from younger flowers. The flower is therefore **protandrous.**

F. GERANIACEAE

Geranium robertianum (Herb Robert).—May - September. The family is not a very large one, consisting only of eleven genera, which are mostly herbs of the temperate zone, with representatives both north and south of the Equator and notably in South Africa. The flowers are regular and constantly have the parts in fives. The fruits are frequently furnished with mechanical dispersal agencies. *G. robertianum* is a very common roadside plant with much divided leaves, and is thickly covered with glandular hairs. The whole plant often has a bright red colour, the amount depending on the degree of exposure to sunshine.

Floral formula : K5, C5, A5 + 5, G(5), ⊕

The **calyx** consists of five pointed sepals, and the **corolla** of five pink petals with veins of darker pink, which may be regarded as guides to the nectar which is found at the base of each of the five outer stamens and collects in five small separate drops. The stamens of the **androecium** are **obdiplostemonous,** that is, they are in two whorls (diplostemonous), of which the outer whorl is opposite the petals, not alternating with them, as would be the case if the normal rule of alternation of successive parts in the flower were observed. There is no obvious explanation for this anomaly. The **gynoecium** produces a fruit of five **cocci** or separable one-seeded portions, each of which is attached above to a segment of the long beak-like style. When the cocci are ripe they split apart suddenly, and the style segments then rapidly roll upwards and throw out the seeds with a motion somewhat like that of underhand bowling.

The flower is protandrous and the five stigmas are kept pressed together during the male phase of the flower, with their receptive surfaces protected, but they usually separate just before the stamens wither, so that there is a short interval during which self-pollination is possible.

G. PAPILIONACEAE

1. ***Pisum sativum*** (Pea).—May-July. The Papilionaceae is one of the largest families of flowering plants and is of world-wide distribution, including almost all types of growth-form.

Floral formula : K(5), C5, A5 + 5, G(2), ·|·

The **calyx** is large and the sepals joined for about half their length into a stout tube which confines the other floral parts and helps to hold them in a fixed relationship to each other, a feature which is unusually well marked in flowers of this type. The **corolla** consists of one large posterior petal, the **standard** or **vexillum,** two lateral petals, the **alae** or wings and two small anterior petals which are completely joined along their lower margins and along their rounded apices, the line of suture being strengthened by a frill of united tissues. These form a pouch, open only on the top, which is called the **carina** or keel.

The **stamens** are united together by their filaments into a tube which fits closely round the ovary. The posterior stamen alone is free, so that the stamen tube is incomplete and through the slit thus left in it the bee can insert his proboscis to the base of the ovary where the honey is secreted.

The alae show a small but rigid pocket-fold near the base, which fits very closely into a corresponding fold in the keel petals. Thus any pressure from the weight of a visiting bee on the alae, which tends to force them down, will also force down the keel. At the tip the free ends of the keel petals overlap, leaving only a small orifice for the stigma.

The **style** is very rigid and stands up at right angles to the horizontal ovary, with its apex curved round towards the centre of the flower, following the line of the carinal suture.

Two folds of the carinal petals lie on the upper surface of the staminal column and keep it in place, while the alae also are held by swellings on the base of the vexillum which press on the base of the alae. Thus all parts are rigidly held in place and return to their correct positions after an insect visit has displaced them. The flower thus preserves its fresh appearance and ensures repeated insect visits.

The stamens lie in two pouches, one on each side of the style, and the pollen is shed in the bud into the conical end of the carina, in contact with the brush of hairs on the stigma. When the weight of an insect presses down the alae the carina is also pressed down, which forces out the stigma through the apical opening of the carina. The stigma has a strong brush of hairs along its upper surface, and this brushes out a mass of pollen from the conical end of the carina. When the pressure is released and the carina rises again, this pollen is rubbed off from the stigmatic brush, and remains outside ready for the next visitor to pick up. The stigma then picks up another load of pollen inside the carina, and is ready to repeat the ejection process. The ends of the stamens likewise press upon the pollen mass in the carina and compact it upwards whenever the carina is depressed.

2. ***Ulex europaeus*** (Gorse).—February - July. The flowers are solitary in the axils of scale leaves. Each has five **sepals,** of which the lateral pair are small and brownish. The anterior-posterior are very much larger and green, but are covered with brownish hairs. The posterior sepal is made up of two, joined together, but sometimes with separate tips. The **vexillum** is folded down over the flower instead of standing upright as in *Pisum*. The **alae** are narrow and are articulated to the petals of the **carina** by a fold near the base, into which fits a corresponding fold of the carina. The latter petals are not actually united, but are closely held together along their upper and lower edges by fringes of interlocking hairs, somewhat like a zip-fastener.

There are two whorls of **stamens** distinguishable, though the filaments are all of them joined into a tube surrounding the ovary. The five outer stamens are longer and thicker than the others, which alternate with them, and they ripen their pollen first, shedding it into the bud. The five smaller stamens then elongate, pushing forward the pollen already shed into the tip of the carina and adding their own to it. Stamens and style together exercise an upward pressure, which is counteracted by the downward pressure of alae and vexillum. When the flower is ready the vexillum expands, and if a bee alights on the alae, their downward pressure is increased to such an extent that the carina petals split apart and the style, with the pollen, jerks upwards explosively, shedding the pollen on the underside of the visitor. As the style strikes the visitor in advance of the pollen cloud it therefore stands a good chance of picking up pollen from some other flower or plant of the species.

The **fruit** is a **legume,** and the seed possesses an oily **aril** or **caruncle** which is said to attract ants and leads to their dispersing the seeds.

H. ROSACEAE

1. ***Fragaria vesca*** (Wild Strawberry).—April - May. The Rosaceae include plants of the most varied habits and of considerable diversity of flower structure, especially with regard to the position of the gynoecium. There is a persistent tendency throughout the family for the floral receptacle to be flattened or hollowed out, either partially or completely enveloping the gynoecium, conditions known as **perigyny** and **epigyny** respectively. This is illustrated by the two examples here described.

In the Strawberry the receptacle has the form of a slightly concave plate with a dome in the centre, while in the Apple the carpels are completely embedded in the receptacle, forming an inferior ovary.

Floral formula : K5, C5, A∞, G∞, ⊕

The leaves have two brownish membranous **stipules** at the base of the petiole, a feature common in the family. This feature is shared by the **sepals,** which also have stipules, though in this case neighbouring stipules have fused together owing to their close proximity. Their double nature is shown by the bifurcate tip. The result is an outer whorl of small green appendages alternating with the sepals and called the **epicalyx.**

The base of the **receptacle** is expanded into a horizontal disc on the edge of which the sepals, petals and stamens are all situated, separated from the gynoecium by a ring-like space which secretes the nectar. The central part of the receptacle rises in the form of a blunt cone, carrying the numerous small carpels, each of which forms an akene. The **torus,** or central receptacle, enlarges and forms the flesh of the ripe " fruit."

2. ***Pyrus malus*** (Apple).—May. The flower differs markedly from that of *Fragaria* in being **epigynous,** *i.e.*, having an inferior ovary. There is a wide range of variation in the Rosaceae in the relationship of the gynoecium to the other flower-parts, and the Strawberry and the Apple present two extremes in this respect.

If a median longitudinal section of the flower be cut, from below upwards, the ovary will be seen to contain loculi (usually five) and the style may be seen leading from the loculi, upwards, to emerge in the centre of a saucer-shaped disc in the centre of the flower. A little above the point of emergence it divides into five lobes crowned with stigmas.

The **sepals** are usually hairy, the **petals** large and pale pink, while the **stamens** are numerous and frequently joined together to a slight extent at the base. All these parts arise on the margin of the disc into which the ovary expands on top, thus being separated by the radius of the disc from the style, which emerges centrally. The flower is **protogynous,** the stigmas overtopping the stamens and ripening before them.

J. SAXIFRAGACEAE

Saxifraga umbrosa (London Pride).—June - July. Other garden species may be used instead, if available. The plants of this family are predominantly herbaceous and distributed all over the world, but chiefly in temperate and alpine regions. Their vegetative appearance is very varied, but very few, such as *Ribes* and *Escallonia*, are shrubby. Flowers are mostly small and hermaphrodite, with the same variation between hypogyny and epigyny which is found in the Rosaceae.

Saxifraga is distinctively an alpine or cold temperate genus, and the species here selected is a very common garden plant. It also occurs (or a very closely related species) at Killarney in Ireland. Hypogyny, perigyny and epigyny all occur in this single genus.

Floral formula : $K5,\ C5,\ A5+5,\ G(\bar{2})$, ⊕

The **sepals** are small and obtuse and are covered, like the pedicel, with red glandular hairs. The **petals** are larger and decoratively spotted with carmine. The **stamens** are about equal to the petals in length. They are separated from the style by a broad and conspicuous **nectary disc,** from the middle of which protrude the pair of **styles.** The **fruit** is a capsule with **axile placentation** and two loculi.

K. UMBELLIFERAE

Anthriscus sylvestris (Hedge Parsley). April - June. The Umbelliferae take this name from their constantly umbellate inflorescences. An **umbel** is a contracted raceme and therefore shows the youngest flowers towards the centre, *i.e.*, the apex. These umbels are usually surrounded by an **involucre** of bracts, and if the umbel is compound, each small umbel has, as a rule, an **involucel** of smaller bracts. The family is made up of rather strong-growing herbs, rich in aromatic oils and usually with hollow stems and much divided, rather fern-like leaves whose petioles, at their bases, embrace the stem. *Anthriscus* is a universal hedge plant, often flowering in extensive drifts of white in sheltered places in April and May. Although the flowers are small they are easily available and quite characteristic of the family.

Floral formula : $K5, C5, A5, G(\bar{2}), \oplus$

The **calyx** is absent or at the most is represented by five minute teeth at the top of the ovary. The **corolla** is white, the petals small and inrolled, while the outer flowers of the umbel are decidedly asymmetrical, their outer petals being much larger than the inner ones, so that the whole umbel appears to be surrounded by a halo of fairly large petals, thus increasing its aggregate conspicuousness.

The **stamens** are five. At first they are bent outwards between the petals so that they are outside the flower. Afterwards, when shedding pollen, they straighten up somewhat, but they are easily broken off altogether by large insects. The gynoecium is peculiar. It is inferior and consists of two uni-ovulate carpels which separate at maturity. Their upper ends are covered by two shining cushions, the **nectaries,** which are the most conspicuous feature in the centre of the flower, and are so fully exposed that the honey is all taken by flies, not bees. At first there are no styles. They only develop slowly as the flower matures, and are not ripe until the stamens have been completely shed.

L. COMPOSITAE

1. ***Doronicum plantagineum.***—April - May. The Compositae are the largest family of the Dicotyledons, including about one in ten of all Angiosperms. They show a very high degree of floral economy and specialisation, and this, combined with extraordinary plasticity of growth form, has contributed to ensure their world-wide distribution in every type of habitat. The characteristic inflorescence, which has given the family its name, is the **capitulum,** a condensed spike, in which the small sessile flowers are crowded side by side on a flattened axis, forming a disc

with the youngest flowers in the centre. The present type represents that section of the family in which the capitulum has two types of florets: (1) the **ray florets** round the margin, with a unilateral, strap-shaped corolla (**ligulate**) which greatly increases the conspicuousness of the whole inflorescence; and (2) the **disc florets** with a minute, regular corolla.

Floral formula : $K0, C(5), A(5), G(\overline{2}), \oplus$

There is an **involucre** of many green bracts round the whole capitulum, rather like the calyx of a true flower. Next come the ray florets. They have no calyx, the corolla forms a long strap developed from one side of the margin of a short floral tube, and composed apparently of three of the full complement of five petals. The stamens are represented only by sterile **staminodes,** but the ovary and style are functional. The disc florets have a regular bell-shaped corolla of five joined segments, at the base of which is a group of fine silky hairs representing the calyx and called the **pappus.** The five stamens are **epipetalous,** and the anthers are joined edge to edge into a narrow tube. The pollen is shed inwards (**introrse**) into this tube and is pushed up and out by the subsequent elongation of the **style,** which has a cushion-like top covered with blunt papillae. The pollen is thus pushed out of the top of the anther tube where it remains until carried away by insects.

The style then elongates further, emerges from the corolla tube and separates into two stigmatic lobes, which curl apart, exposing their receptive surfaces. An insect, therefore, walking across the disc inevitably carries pollen from the younger flowers to the older and effects cross-pollination. As the pollen is all shed before the stigmas open there is probably little self-pollination.

Doronicum has been chosen as a type because it is the earliest of the large-flowered Composites to come into flower, but there are very many possible alternatives, from the humble Daisy upwards.

2. ***Taraxacum officinale*** (Dandelion).—March - October. This type of inflorescence differs from that in the Daisy section of the Compositae in having all the florets of the capitulum similar and all ligulate, so that there is no disc. All the florets therefore resemble in appearance the ray florets of the other section.

There is a double **involucre** consisting of an outer group of **recurved bracts** and an inner group of **upstanding bracts** which closely surround the mass of florets, and in the bud stage overtop and protect them. The individual florets have no bracts and are closely set on an inflorescence disc which is first concave, later flat and finally convex. As a capitulum is a condensed spike, *i.e.*, a raceme of sessile flowers, the youngest flowers are at the centre (apex) of the inflorescence.

Each flower is tubular, with a minute whitish **ovary** at its base, above which rises a short columnar stalk bearing the **calyx,** which is entirely transformed into a mass of silky hairs called the **pappus,** and the tube of five joined **petals** which splits down one side as it grows, so that finally it stands off to one side of the floret, overtopping the sexual parts and terminated by five teeth representing the free tips of the petals.

Inside the corolla are five **stamens** joined by very short filaments to the petals. The anthers are also joined firmly edge to edge to make a second, **staminal,** tube. The **gynoecium** consists of the unilocular one-seeded **ovary** at the base of the floret and the style, with two long stigmatic lobes at the top, which rise in the centre of the flower. The two stigmas are at first closely pressed together and non-receptive, but the outside of each lobe is covered with numerous hairs which act as a pollen brush, sweeping it out of the anthers as the style grows up the middle of the stamen tube with an action exactly like a sweep's brush going up a chimney. The stamen tube protrudes some millimetres above the corolla tube, and the style protrudes about another 5 mm. beyond that. After the style has emerged the stamens remain together, the pollen being attached all round the style, and accessible to small insects walking across the capitulum. The nectar fills the corolla tube, and so is accessible to short-tongued insects. The capitulum closes up at night and in wet weather, so completely that only the involucral bracts remain visible, thus effectively protecting the pollen, but when expanded it is so conspicuous that it is visited by a great variety of insects. Nevertheless, it retains the possibility of self-pollination. The stigma lobes only separate and expose the receptive surfaces after most of the pollen has been removed, but they curl backwards until they touch the pollen-bearing region of the style, and observation shows that they readily pick up some of the remaining pollen of their own florets in this way. Cross-pollination may, however, be brought about by insects walking from the younger central florets towards the older ones near the margin. (But see p. 451).

M. ERICACEAE

Erica cinerea (Heather). July - September. The family is one of woody plants, mostly small shrubs, but in *Arbutus* (Strawberry Tree) and *Rhododendron*, reaching the size of trees. The distribution is mostly cold-temperate and arctic, but *Rhododendron* is most at home in alpine regions of warmer latitudes, especially the Himalayas. In the far north, beyond the tree limit, the Ericaceae become dominant on the wind-swept tundras, and are indeed almost the only woody plants found there.

The family is remarkable for the formation of **mycorrhiza,** the symbiosis with a root-fungus being in many cases obligatory, as in the Orchids.

The flowers are mostly bell-shaped and the anthers open by **apical pores.** Heather is characteristic of acid peaty soils, especially in the maritime zone of North Europe.

Floral formula : $K4, C(4), A4+4, G(4), \oplus$

The **sepals** are narrow and linear like the vegetative leaves. The **petals** are completely united, except at the tips, and form a little pendant bell with a rather narrow mouth which is blocked inside by the ring of anthers closely surrounding the single style. The **stamens** are obdiplostemonous. Each **anther** is composed of two loculi only, with oblique apical openings and from the base spring spur-like **appendages** directed outwards. Below the ovary is a ring-like **nectary.** If an insect presses its proboscis into the flower to get at the nectar, it is bound to pass between the spurs of the anthers, which are thus pulled away from their position around the style, and the pollen liberated. The flower is protandrous. When the stigma is ripe, its position at the mouth of the flower tube enables it to pick up the pollen from the proboscis of an insect coming from a younger polleniferous flower.

The **ovary** forms a four-celled **capsule** which opens **loculicidally,** releasing numerous very small seeds containing **endosperm.**

N. PRIMULACEAE

Primula vulgaris (Primrose).—April - May. Another predominantly herbaceous family, with a cold-temperate distribution, many being alpine or arctic. The flowers vary greatly, but are all sympetalous and typically pentamerous, with epipetalous stamens and capsule fruits. The genus *Primula*, with three hundred species, forms about one-half of the entire family.

For description of the flower of *Primula vulgaris* see page 239, and for the pollination see page 251.

Floral formula : $K(5), C(5), A5, G(\underline{5}), \oplus$

O. SOLANACEAE

Solanum tuberosum (Potato).—June - August, or *S. dulcamara* (Bitter-Sweet) June - August. The Solanaceae stand very near the Scrophulariaceae, but differ from them in the regular symmetry of their flowers, in the general presence of five stamens and in the oblique position of the septum in the ovary, a small but constant feature. They are on the whole somewhat poisonous in character, due to the number of specific alkaloids they produce.

Potato and Bitter-Sweet flowers are very similar, and either of these or *S. **nigrum*** (Nightshade) may be used.

Floral formula : $K(5), C(5), A5, G(\underline{2}), \oplus$

The **sepals** are small, green and partially joined into a bell-shaped tube. The **petals** are also joined into a short basal tube inside that of the sepals, the upper part of each petal being, however, free and reflexed. The **corolla** is **valvate,** that is, the petals do not overlap each other (imbricate) but are joined edge to edge. The **stamens** are set at the edge of the corolla tube. Each has a short filament and a large yellow anther which stands close to the style. The **gynoecium** is composed of two carpels with a single style which is longer than the stamens and often deflected downwards. The fruit is a **capsule.** No nectar is formed and few insects visit the flowers. As the downward bending of the style places the stigma below the stamens, self-pollination must be the normal result.

P. SCROPHULARIACEAE

1. ***Digitalis purpurea*** (Foxglove).—July - September. This family is a very large assemblage of species which agree in their zygomorphic tubular flowers with five stamens, often suppressed in part, and a bicarpellary ovary with a transverse septum, which develops into a dry capsule. The family is world-wide in distribution and includes plants of every sort of habit. The Foxglove, though it flowers rather late in the season, is the best available type of the tubular flowered Scrophulariaceae.

Floral formula : K5, C(5), A4, G($\underline{2}$), ·|·

The **sepals** are free and unequal, the posterior one smaller than the other four. The **corolla** forms a long saccate tube with five rounded lobes at its mouth, which represent the five petals composing it. The upper and lower petals are larger than the laterals, the lowest being both longer and larger than the other four, and giving the tube its pouched appearance. There are four **stamens,** the posterior (fifth) being suppressed. The lower pair are longer than the upper pair, and all four are bent upwards so that they lie against the top of the floral tube. The **style** is simple, rather longer than the stamens and also pressed against the top of the tube behind the stamens.

The stamens ripen before the expansion of the two stigmatic lobes at the tip of the style. Cross-pollination is the work of humble bees, the only native insect visitors which are large enough, but self-pollination probably frequently occurs.

If *Digitalis* flowers too late in the year to be used as a type in class work, a useful substitute may be found in *Linaria cymbalaria*, the Oxford, or Wall Toad-Flax, whose light-purple flowers are out by the end of April. They are small, but closely resemble the Snapdragon of late summer. From *Digitalis* they differ chiefly in the **personate** perianth, that is to say, the mouth of the floral tube is closed by two large yellowish hummocks formed by upward pouching of the three lower petals. This prevents the nectar being stolen by insects which are too small to benefit the plant by effecting pollination. Only a strong insect can force the mouth open, by squeezing it between his front legs, and thus obtain the nectar, and the flower is, in fact, chiefly visited by bees. The nectar is held in a short spur produced by the lower petal. Stamens and carpels are as in *Digitalis.*

2. ***Veronica chamaedrys*** (Germander Speedwell).—April - July. The flower of *Veronica* differs from that of the Foxglove very markedly in general appearance, but nevertheless there is a similarity in the fundamental construction.

Floral formula : K(4), C(4), A2, G(2), ·|·

The **calyx** has only four sepals, joined at the base, the posterior (fifth) sepal being suppressed. The **corolla** has four petals, the posterior being double the breadth of the others and probably representing two fused together. The petals form only a very short tube at the base, the greater part of each being free. There are only two **stamens,** the lateral pair, the others being suppressed. The **gynoecium** has the typical bilocular structure, with a transverse septum, which is characteristic of the family. It forms two flattened capsular segments like purses, each containing several seeds.

Q. LABIATAE

Lamium album (White Dead Nettle).—May - December. The Labiatae are mostly herbaceous, though a number of shrubs and trees are known. They are predominantly hairy, glandular and with opposite leaves. They are mostly inhabitants of dry situations in the temperate regions, and their main area is round the Mediterranean. The flowers are zygomorphic, and the name of the family is due to the two-lipped appearance produced by the division of the corolla tube into an upper and lower segment, composed of two and of three petals respectively. The family is closely related to the Scrophulariaceae, differing chiefly in the fruit, which is, in this family, divided into four one-seeded portions or **nutlets.**

Lamium album is a common roadside weed, flowering at almost all times of the year but chiefly in early summer. The flowers are borne apparently in whorls in the axils of large **leafy bracts,** but these whorls are really formed of two very much **condensed cymes** (dichasia), one in the axil of each bract.

Floral formula : $K(5), C(5), A4, G(\underline{2}), \cdot|\cdot$

The **calyx** is a short green tube with five sub-equal teeth. The **corolla** is white and slightly woolly, tubular below and divided above into two segments, the upper hooded, covering the stamens, and the lower formed of three petals, of which the two lateral ones are reduced to mere points. The **androecium** consists of four stamens, parallel and ascending at the back of the flower under the upper lip, to which they are attached. The **anthers** have only two pollen loculi. The **gynoecium** is formed of two carpels, but a division early separates these into four, each segment containing one basal ovule, with the **style** arising from the base of the ovary **(gynobasic),** in the centre of the four segments. Each segment forms a one-seeded hard fruit called a **nutlet,** which is shed separately after the corolla has dropped off and taken the stamens with it.

This is a bee flower, for only long-tongued insects can reach the nectar, which is secreted by a fleshy lobe on the front of the ovary and fills the narrow lowest portion of the corolla tube. This tube is lined upwards with hairs which exclude small flies.

Bees land on the lower lip of the flower and thrust the thorax into the corolla opening, thus rubbing their backs against the stamens and receiving pollen. The style ends in a two-lobed stigma, one lobe projecting downwards from beneath the hood of the corolla. This projecting lobe touches the backs of visiting bees and picks up pollen which they have brought with them. Self-pollination may also sometimes occur by the same means.

R. BETULACEAE

Corylus avellana (Hazel). February - March. Male and female flowers occur on the same trees, but in separate groups. The **male inflorescences** or **catkins** are formed in autumn near the ends of the year's shoots. They remain dormant for several months, but in early spring they begin to elongate and are 2 to 3 in. long when ripe. The **female catkins** are very small and are formed from axillary buds somewhat farther down the shoots. The internodes are suppressed so that the female flowers remain enclosed by their bracts, only the red-coloured **stigmas** protruding.

The male catkins are made up of sessile male flowers, each subtended by a **bract** with two adherent **bracteoles.** There are eight stamens, in four pairs, attached to the bract, which overtops them.

The female catkins are very short. Every bract subtends a pair of female flowers, in each of which is an ovary surrounded by a minute membranous **perianth** and producing a very short style which branches into two long reddish stigmas which protrude above the bracts.

The plant is wind pollinated (**anemophilous**), the pollen being caught by the long stigmas of the female. Cross pollination is assisted by **dichogamy,** some plants being protandrous, others protogynous, while both types may occur on neighbouring trees.

S. SALICACEAE

Salix caprea or ***S. atrocinerea*** (Common Pussy-Willow). April - May. The flowers of this family, which includes only the Willows and the Poplars, are arranged in close racemes known as **catkins,** the sexes being separated on different individuals. This is called **dioecism,** and it is not very common in the plant kingdom.

The flowers are individually very small and inconspicuous, but the mass of flowers in a catkin has a collective conspicuousness, especially in the early spring, when they appear, which attracts many insect visitors. Although there are no coloured petals the male flowers offer an abundance of pollen, and both sexes possess nectaries which are much appreciated by bees before the fruit blossom season.

The **male catkins** are comparatively showy with their yellow anthers. The individual flowers are of the simplest. Each has a small brown **bract** fringed with silky hairs or **cilia.** In its axil are two stamens with long filaments, at the base of which is a little truncated cone, slightly hollowed on top, which is the **nectary.**

The **female flowers** also possess a ciliated bract, in the axil of which arises a stalked gynoecium consisting of two united carpels which terminate in two long stigmas. A transverse section of the ovary shows that there is only one loculus with numerous ovules arranged on two longitudinal placentae. When the seeds are ripe the carpels separate down the suture of junction between them, and the minute seeds escape. Each seed is provided with a tuft of fine hairs, which are outgrowths of the testa (**arils**). These enable the seeds to ride on the wind for considerable distances.

The female flower also has a **nectary,** larger and more conspicuous than in the male, but of the same stumpy form, with a slightly hollowed apex.

MONOCOTYLEDONS

T. ARACEAE

Arum maculatum (Cuckoo Pint).—April - May. The inflorescence, which simulates a large flower, is a spike or **spadix** enclosed in a green sheathing bract, the **spathe,** considerably larger than the inflorescence itself. The latter is **monoecious,** with a zone of female flowers round its base, above which is a zone of male flowers separated by an interval from the females and followed again by a zone of abortive flowers, of which the apices are elongated like hairs and point downwards. Above this is the sterile club-shaped appendage of the spadix, purple and conspicuous, as it stands framed in the unfolding spathe. The functional **female flowers** are reduced to single ovaries with no other floral parts whatever. Each ovary is a short ovoid structure, creamy coloured and having a round sessile stigma. It contains two rows, each of two to three ovules. The anthers which constitute the **male flowers** are purplish. There are no male perianths either, but if several anthers are removed with a needle it will be seen that they are associated together in pairs on a common base. Each male flower therefore consists of two stamens. Each stamen has four pollen loculi. Between the female and the male zones and again above the male zone, are a number of abortive florets with tail-like appendages about 5 mm. long. The spathe is wide open above, but the margins overlap at the base, and the spathe is here enlarged into a round chamber enclosing the flowers, which connects to the open spathe above by a narrow waist-like portion, just at the level of the upper zone of sterile florets, so that the passage is partially blocked by their appendages. The fruits are berries with a bright orange colour, born in a close spike.

This is an example of extreme floral reduction, combined with a very efficient pollination mechanism (see p. 250).

U. ORCHIDACEAE

Orchis mascula (Early Purple Orchis).—April - June. The Orchidaceae is one of the most remarkable families of flowering plants. The exceptional degree of specialization of their flower structures to insect pollination is perhaps their most striking feature, but they also present a very interesting and close dependence on **mycorrhiza,** *i.e.*, a symbiosis with root-inhabiting fungi, and have an almost world-wide range, combined with great diversity of ecological characteristics. *Orchis mascula* is the first to appear in our spring woods, and its large flowers make observation easy.

Floral formula : K3, C3, A1, $G(\bar{3})$, ·|·

The **calyx** and **corolla** are both petaloid and of a similar bright purple colour. As the flower is turned over during development through 180° (**resupinate**), the large lower petal or **labellum** of the mature flower is really the posterior or uppermost petal. It differs from the others in being expanded and serving as a landing-place for insect visitors. It is also marked with conspicuous dark spots on a lighter background, near the throat of the **spur,** into which it is prolonged backwards. This spur is the **nectary,** and is sometimes almost full of nectar. The other two petals and the three sepals make a hood across the back of the flower. Immediately above the entrance to the spur is a sticky-looking patch with a narrow membrane down each side. This is the **stigma.** If one looks round to the back of the flower it will be seen that this stigma lies immediately on top of the junction between the **ovary,** which acts as a stalk, and the flower. There are three **carpels** in this ovary, and the functional part of the stigma is made up of the stigmas belonging to two of these carpels. The stigma of the third carpel is changed into a tiny red pouch, the **rostellum,** which protrudes above the stigma proper. The rostellum lies at the foot of the single **stamen,** which stands immediately under the hood of the perianth. The stamen consists of two distinct half-anthers, in each of which is a double **pollinium,** or mass of pollen grains, stuck together in small packets on a viscid framework which holds them together. The viscid material is prolonged downwards into two legs which end in two sticky discs inside the rostellum pouch. At a touch from outside the latter slips downwards and backwards, leaving the discs or **retinacula** to adhere to whatever object has caused the disturbance. This is usually the head of a honey bee. When it withdraws, the pollinia are drawn out of their anthers. They come out easily, as the anthers have dehisced longitudinally when ripe, and the pollinia thus are carried off, adhering to the bee's head. During the flight of the bee to another flower the legs of the pollinia dry and contract, so that the pollinia are drawn down out of the original vertical position and point forwards. This takes fifteen to thirty seconds. When, therefore, the bee enters another flower the pollinia are now in position to make contact with the stigma, to which they adhere, masses of pollen being torn off and retained by the stigmatic mucilage. Cross-pollination is thus assured, while the relative positions of the organs in the flower makes self-pollination practically impossible. The ovary is unilocular and has three parietal placentas bearing an enormous number of minute seeds. These have a loose papery testa and are dispersed by wind.

V. LILIACEAE

1. ***Tulipa,*** any garden type (Tulip).—April - May. The flowers are all regular, with a perianth in which all the parts are petaloid and rarely distinguishable as calyx or corolla. The flowers are among the largest and handsomest of all and constitute a central type among Monocotyledons.

The Tulips of gardens are the offspring of hybridization between a number of species, mostly from the Near East, and they have been in cultivation for over three hundred years.

Floral formula : $P3+3, A3+3, G(\underline{3})$, ⊕

The **perianth** is large, showy and petaloid, but the flower produces no nectar, so insect visitors are not abundant and come only for the pollen. The **stamens** are large and the pollen usually dark-coloured. The filaments are exceptionally stout and rigid. The anthers are fixed only by one point of their bases to the tip of the filament, so that they can swivel round through 180° or more. The **gynoecium** consists of three joined carpels, with separate loculi, forming a stout column capped by three stigmatic pouches, the lips of which form prominent ridges, one over each loculus. Placentation is axile and the fruit is a capsule.

2. ***Scilla non-scripta*** (Bluebell).—April - June. This plant, although its flowers are picked by thousands every year, does not appear to diminish in numbers, partly due to the ready germination of its seeds and partly to vigorous vegetative reproduction by the bulbs.

Floral formula : $P3+3, A3+3, G(\underline{3})$, ⊕

The flower is a simple structure. There are six **perianth segments** alternating in two whorls of three and practically free from each other, but there is nothing to show whether they should be regarded as sepals or petals. All are alike and all petaloid. They often show a slight spiral twist in the opening flower.

The **stamens** are attached for about half their length to the perianth members. The **gynoecium** consists of three carpels joined into a compound ovary with a single style and stigma. This ovary stands on a short pedicel and the nectar gathers in the space below it. The fruits are capsules.

W. AMARYLLIDACEAE

Narcissus pseudo-narcissus or other garden species (Daffodil).—March-April. The flower is regular with a perianth in which all the parts are petaloid. They are in two whorls but are closely united to form a long floral tube. At the mouth of this tube there is a ring-shaped outgrowth known as the **corona.** In some species this is no more than a frill but in others, as in the common Daffodil, the corona is enlarged into a trumpet which is the most conspicuous part of the flower.

There are six stamens, arranged in two whorls. The ovary is inferior and trilocular and gives rise to a capsular fruit.

Floral formula : $P(3+3), A\ 3+3, G(3)$, ⊕

In the Poets' Narcissus the tube is narrow and the corona is small, but coloured an intense orange-red which contrasts strongly with the background formed by the perianth segments, three sepals and three petals, all of which are alike petaloid and white.

X. GRAMINEAE

Arrhenatherum elatius (False Oat Grass).—June - July. The grasses are the most widely spread and probably the largest of the families of the flowering plants. They are highly specialized in their anatomical structure and extremely adaptable ecologically, yet they maintain throughout the principle of **anemophilous pollination,** which is clearly a wasteful and reputedly a primitive method. Their peculiar floral structure, however, succeeds in minimizing the disadvantages of the method.

Arrhenatherum is one of the largest native grasses, abundant in every hedgerow, and its flowers, when found wide open early on a summer morning, display the grass flower structure at its clearest.

Floral formula : $P2, A3, G\underline{(2)}$, ·|·

Each flower is subtended by two scale-like structures, the lower of which is simple and is called the **flowering glume,** the upper is double and is called the **palea.** These are presumably bract and bracteole respectively. The **perianth** is represented only by two minute scales, the **lodicules,** which represent the two anterior petals, the posterior petal being suppressed. The **stamens** alternate with the lodicules and have long delicate filaments with loosely swinging (**versatile**) anthers which protrude freely from between the bracts. The two **carpels** are completely united, with only one loculus and one ovule, but with two very large feathery stigmas which, like the anthers, protrude from the flower.

The flowers are arranged in close groups or **spikelets** which are really small racemes subtended at their base by a large bract called the **barren glume.** In *Arrhenatherum* the spikelet contains only two flowers, but this is exceptional. The flowers are protandrous, so that the lower flowers of the spikelet are pollinated by pollen dropped directly from the anthers of the late-developing upper flower. Self-pollination is the rule and, as it takes place chiefly in the still air of early morning, there is comparatively little waste of pollen.

PART SEVEN

PLANT PHYSIOLOGY

Hydrogen Ion Concentration: The Measurement of *p*H

For simple estimations, and indeed for most biological purposes, the indicator method of measuring *p*H is quite satisfactory, while it is much quicker and less expensive than the electrometric methods.

A selection of indicators is required which covers as much as possible of the *p*H scale. If expense must be limited then one may confine oneself to those near the neutral point, *p*H 7·0, as reactions lying near this point are the most general in biological systems.

The following are suitable :—

Indicator.	Range *p*H.	Colour Change, Acid to Alkali.
Thymol Blue (acid range) .	1·2— 2·8	Red-Yellow.
Bromo-phenol Blue . .	2·8— 4·6	Yellow-Violet.
Methyl Red . . .	4·2— 6·3	Red-Yellow.
Bromo-thymol Blue . .	6·0— 7·6	Yellow-Blue
Phenol Red . . .	6·8— 8·4	Yellow-Red.
Thymol Blue (alkaline range)	8·0— 9·6	Yellow-Blue.
Thymol Violet	9·0—13·0	Yellow-Green-Violet.

Make up a 0·4 per cent. solution of each in absolute Alcohol. These solutions will have the un-ionized (acid) colour of the indicator. The colour change of each indicator is completed within the range of *p*H shown, and at intermediate points of the range intermediate tints will be seen. By matching these against standards any *p*H within the range of the indicator may be ascertained. If the *p*H of a liquid is entirely unknown it is advisable to use a mixed, or " universal " indicator, which will show approximately in which part of the scale the unknown *p*H lies, and therefore which indicator to use for final measurement.

Such a Universal Indicator may be bought or made up. A receipt is given in the Appendix (p. 460).

The standards used are made up with buffered fluids, to which the same proportion of indicator is added as to the unknown. Universal buffers may also be purchased, with instructions for making them up to any given *p*H, but the mixtures on the following page will be found especially useful as they cover the common biological reactions :—

Sodium phosphate Buffer—

0·1 M NaH_2PO_4 . .	10	8	6	4	2	0 c.c.
0·1 M Na_2HPO_4 . .	0	2	4	6	8	10 c.c.
*p*H of mixture . .	4·0	6·0	6·5	6·75	7·2	9·0

To 10 c.c. of the unknown and 10 c.c. of the buffered standard[1] add equal amounts (about 10 drops) of indicator and compare. Intermediate buffers may be made up by using intermediate proportions, as determined graphically. Buffer standards will keep in the dark for two to three months if well corked, or for considerably longer if the tubes are hermetically sealed. The Sorensen Phosphate Buffer Solutions are given in the Appendix (p. 462).

Liquids may also be brought to a predetermined *p*H by selecting a standard of the required value, adding indicator and titrating the unknown with Hydrochloric acid or with Sodium hydroxide, as required, until the colours match in both tubes.

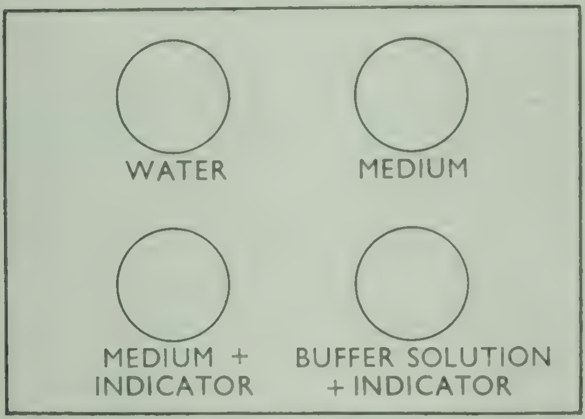

FIG. 61.—Diagram of the arrangement of the comparison tubes in *p*H determination.

In the case of coloured unknowns, four comparison tubes must be arranged as in Fig. 61. The tubes must be screened in some way, *e.g.*, by placing them in holes in a block of wood, as shown by the outline in the figure, which is slotted or bored horizontally to allow transmitted light. Comparison is greatly aided by this precaution.

Section 1. Colloidal Phenomena

A. LYOPHILE SOLS

1. Weigh out about 1 gm. of dry starch and mix it to a cream with a little cold water. Boil about 200 c.c. of water in a beaker and pour in the starch mixture. Continue boiling for a few moments, stirring well. The starch is **lyophile,** *i.e.*, its particles readily take up water and become hydrated, so it forms an opalescent emulsoid **sol.**

2. Weigh out about 5 gm. of white flour and mix thoroughly with about 100 c.c. of cold water. Allow to stand for two hours and then filter. The filtrate is an albumen sol which froths on shaking.

B. EMULSIONS AND LYOPHOBE SOLS

1. Fatty oils are practically insoluble in water, but they can be made to form permanent **emulsions** if they are sufficiently finely divided. Only small quantities, however, can be thus emulsified.

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[1] Hard glass test-tubes (*e.g.*, Pyrex) must be used or alkalis will dissolve from the glass.

Take a drop of Olive or Castor Oil in a test-tube and half fill the tube with absolute Alcohol. Shake well and pour into a large beaker of water. A fine white emulsion of oil in water will be formed, because by this means the oil is very finely dispersed throughout the water and forms a true colloidal system.

2. If the oil be partly saponified before dispersal, the soap, which readily absorbs water, forms a coating on the oil particles. In this way the surface tension between oil and water is greatly reduced, and much larger amounts of oil may be emulsified without precipitation, the soap acting as a stabilizer.

Take equal amounts of Olive Oil in two test-tubes and add equal amounts of water to each. To one tube add a few drops of Sodium hydroxide solution. Shake both tubes. Only that containing the alkali will form a permanent dispersion in the water. Note that soaps "protect" or stabilize otherwise unstable emulsions. They play this part for the oils in protoplasm.

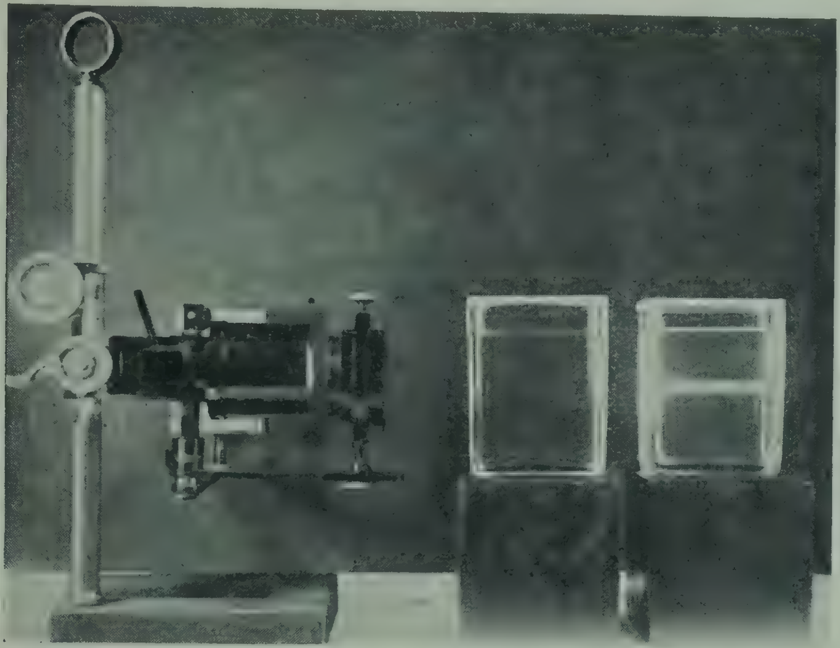

FIG. 62.—Apparatus to demonstrate the Tyndall effect (see p. 294).

3. Even solid insoluble substances can form colloidal suspensions if they are sufficiently finely divided. This can be accomplished either physically, *i.e.*, with metals, by electric sparking under water or by special chemical means.

Take 5 c.c. of a 1 per cent. solution of Silver nitrate and add dilute Ammonium hydroxide drop by drop until the precipitate which is formed at first just disappears. Then dilute with 100 c.c. of distilled water and pour into a beaker.

Make up 100 c.c. of 0·5 per cent. Tannic acid solution and add this to the Silver nitrate solution in the beaker. A suspensoid sol of metallic Silver is formed, which is brown by transmitted and green by reflected light.

4. Make up a 33 per cent. solution of Ferric chloride in water and pour 5 c.c. of the solution into 500 c.c. of boiling distilled water in a beaker. A suspensoid of Ferric hydroxide is formed, which appears clear, but of a deep brownish-red colour. That this is not a simple solution of Ferric chloride is shown by the fact that it is immediately precipitated by Sodium sulphate, which would not normally affect either Ferric or chloride ions.

5. Although a sol may appear clear to the eye when viewed by ordinary light, a beam of concentrated light passing through it will show the presence of a cloud of ultra-microscopic particles, especially when viewed against a dark background. This is a similar effect to that shown by a bright beam of light in a dark room, since the atmosphere is also a suspensoid of colloidal-sized particles in a gaseous medium.

6. Try this effect with the Ferric hydroxide sol above, using, as a standard of comparison, a beaker of distilled water which has been allowed to stand for some time. The beam from an ordinary projection lantern is suitable, or a narrow sunbeam, if available, reflected from a mirror. The appearance in the sol is called the **Tyndall effect,** and is common to all colloids (Fig. 62). Examine the reflected light from the colloid particles with a Nicol prism or a Polaroid disc. It will be found that it shows polarization.

C. FLOCCULATION OF COLLOID SOLS

If the particles of a colloid are negatively charged they can be flocculated (precipitated) by adding an excess of positively charged ions (kations) and vice versa. These ions are active roughly in proportion to their valency, in the ratio $x : x^2 : x^3$, etc. The mobility of the ions is, however, important, so that the H ion, although only monovalent, is highly active.

1. Take a piece of china clay about the size of a pea and work it into a paste with about 5 c.c. of water. Add this to about 200 c.c. of distilled water containing a trace of ammonia, and shake strongly in a flask. Put aside to settle till required. The clay particles produce a negatively charged suspensoid.

Make up the following solutions of equal anionic concentrations :—

Substance.	Strength of Solution.	Molecular Wt. (M.)	Valency.
Potassium chloride . . .	0·4M	74·5	1
Calcium chloride . . .	0·6M	111	2
Aluminium chloride . .	0·8M	135·5	3

Make dilutions of these three in the following proportions to water—1 : 1, 1 : 4, 1 : 9. Place exactly 9·0 c.c. of clay sol in each of the ten test-tubes. To one of each of *nine* of these add 1 c.c. of one of the nine salt dilutions and label the tubes. To the tenth tube add 1 c.c. of decinormal Hydrochloric acid to supply free hydrogen ions.

Stand for two hours and observe the rate of settling of the clay in each case, consequent on the aggregation of the colloidal clay particles. Calcium salts in the soil operate in the same way in flocculating the clay, and thus " opening " the texture of the soil.

2. If the colloid is positively charged it may be flocculated by an excess of anions with negative charges. The valency rule applies here also.

Test the Ferric hydroxide sol in the same manner as above, using solutions of three different anions :—

Substance.	Strength of Solution.	Molecular Wt. (M).	Valency.
Potassium chloride . . .	0·4M	74·5	1
Potassium sulphate . . .	0·6M	174	2
Potassium ferricyanide . .	0·8M	329	3

D. BROWNIAN MOVEMENT

The activity of fluid and gaseous molecules communicates itself to dispersed particles if they are sufficiently minute. These particles behave physically as if they were themselves large molecules. Their mean path of oscillation varies inversely as the square root of their radius and as the square root of the viscosity of the medium.

Mix a small quantity of either Gamboge or Carmine powder with water and mount a drop on a microscope slide, diluting with water if necessary. Diluted Indian ink often gives good results, or a drop of the Olive-Oil emulsoid previously made may be used. In the latter the smallest droplets, though liquid, will show good movement.

Examine with the highest magnification available.

A suspension of bacteria also shows Brownian movement, which must be distinguished from the vital motility shown only by some species.

E. EFFECTS OF REACTION (*p*H): AMPHOTERIC COLLOIDS

Some colloids, particularly proteins, may carry either positive or negative charges, according to the hydrogen ion concentration of the medium. In more acid media they are positive, and in less acid or alkaline media, negative. They are called **amphoteric.** The *p*H at which the particles are electrically neutralized is called the **iso-electric point.**

Make up a sol of white of egg (albumen) with four times its volume of distilled water to which a single drop of strong Ammonium hydroxide has been added. To a sample of this sol add dilute Acetic acid, drop by drop. A maximum of opalescence (flocculation) will be observed, followed by a decrease at higher acidities. This maximum corresponds with the iso-electric point of the protein. Preserve the original sol for comparison below.

Compare the albumen at the iso-electric point with the original sol in respect of (*a*) viscosity, (*b*) coagulability, *i.e.*, gel formation.

(*a*) *Viscosity.*—Suck up the fluid into a narrow-bore glass tube and time the rate of fall over a measured length of the tube. The time taken is directly proportional to the viscosity, when the fluids have the same density.

(*b*) *Coagulability.*—Heat samples of the two sols in test-tubes immersed in a water bath and take the temperatures at which coagulation (gelation) begins.

Using a " Universal " indicator, test the *p*H of the two sols.

At the iso-electric point the colloid particles have their maximum density, minimum hydration and minimum volume. Thus the system has its minimum viscosity and maximum coagulability at the same point.

Changes of reaction towards the iso-electric point cause contraction in the volume of the colloid. This is the basis of both protoplasmic and muscular contraction. If two proteins have different iso-electric points the same change of reaction may lead to contraction in the one case and swelling or solation in the other. A case in point is the solution of the nuclear membrane at the same time that the chromatin is condensed into chromosomes.

F. IMBIBITION

1. Place about 2 gm. of dry starch in a small beaker. Add water gently and stir *carefully* with a thermometer. Note that there is a rise in temperature due to the imbibition of water by the starch. Imbibition, therefore, means the performance of molecular work and the release of energy.

2. Use a thread of agar as material to illustrate the swelling properties of a gel due to imbibition. Agar is a carbohydrate gel obtained from Red seaweeds and mostly imported for bacteriological and medicinal use.

Tie one end of a thread of agar about 6 in. long to a lead weight and connect the other end by means of a twisted rubber band to a very light lever with a sharp point, such as a glass thread. Arrange the fulcrum so that the lever will give a magnification of movement of at least ten times and, if necessary, attach another small weight to the long end so as to keep a slight tension on the agar thread.

Lower the weighted end of the agar into a glass jar or cylinder and fix a vertical scale to measure the movement of the lever.

Fill the jar with water at room temperature and note the extension of the agar as the water is imbibed.

Repeat the measurement by pouring off the water and filling up with water at 25°, 35° and 40° C. successively. Plot the successive expansions of the thread. Notice whether the effect of successive temperature increases is linear.

3. The different behaviour of a protein gel and a carbohydrate gel with different iso-electric points, in imbibing water, in relation to the effect of hydrogen ion concentration may be illustrated by comparing gelatine and agar.

Cut small cubes of 25 per cent. gelatine and 5 per cent. agar respectively. Weigh. Place them in tubes of the following mixtures :—

N/10 Sodium hydroxide	N/10 Phosphoric acid
0 c.c.	30 c.c.
5 c.c.	25 c.c.
12 c.c.	18 c.c.
15 c.c.	15 c.c.

Keep for several hours in a cool place, then weigh the cubes. Test also the *p*H of the above buffer mixtures.

4. The colloidal material in seeds imbibes water during germination, and is capable of exercising very great swelling force in consequence.

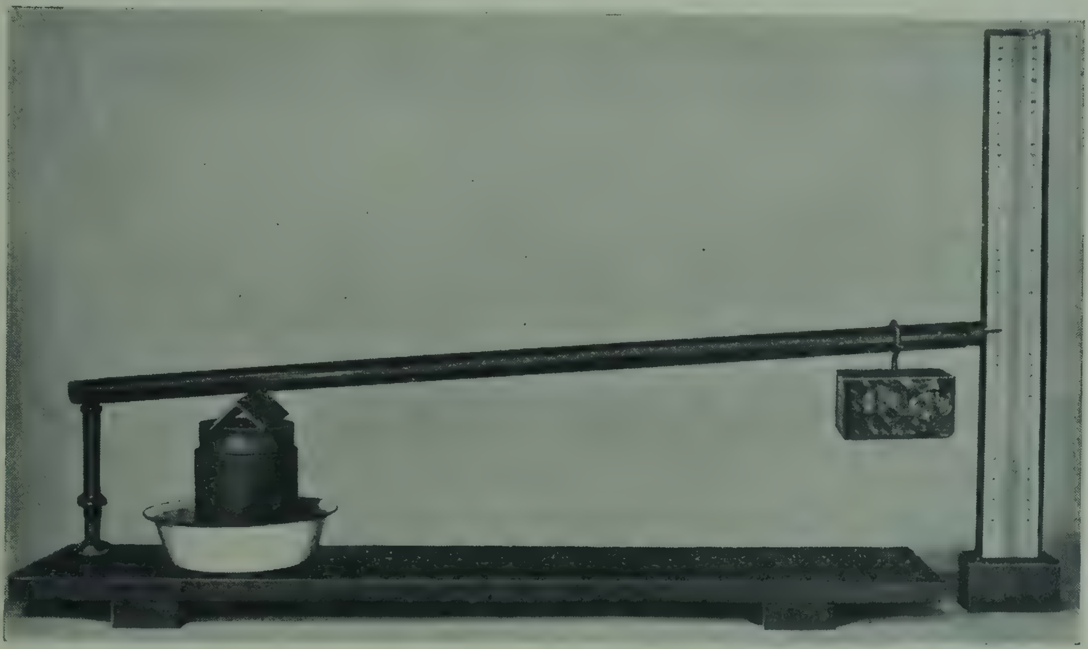

FIG. 63.—Apparatus to demonstrate the swelling power of seeds.

Take an ordinary 8 oz. medicine bottle and fill it with dry seeds : Peas, Lentils or Haricot Beans are all good. Pack them in tightly, leave the neck open and place some rubber bands around the bottle to hold it together. Plunge the whole under water in a deep basin. Water is taken in by the seeds and the bottle is burst by their swelling pressure.

5. To measure the force exerted in swelling, fit up the following apparatus (see Fig. 63). Obtain a 9 in. by 1 in. board 3 ft. long. Close to one end screw in a ring bolt about 6 in. long and get a stout iron rod or iron gas tubing about a yard long, which will pass easily through the ring of the bolt. This provides a lever.

Select a cylindrical tin about 3 in. across and drill a number of small holes in the sides close to the bottom. Fit another tin, or preferably a cylindrical block of wood to slide loosely inside the first tin and of the same height.

Place about an inch of dry Peas in the bottom of the perforated tin, cover them with the block of wood, stand the whole in a shallow basin of water and place it under the lever, close to the ring bolt. The lever should rest on a piece of angle iron or on another short length of iron tube which is placed crosswise on top of the block. Heavy weights may now be hung on the lever, or a spring balance may be fixed so that an upward movement of the lever will stretch the spring. If weights are used a scale must be fixed to show the upward movement of the lever as the seeds swell. In this way very heavy pressures may be applied to the seeds.

If $ab =$ distance from bolt to fulcrum, and $ac =$ distance from bolt to weight, then $\frac{ac}{ab} \times$ weight $=$ the pressure on the seeds. Add to this the pressure of the lever itself. If af is the length of the lever, and $w =$ its weight, then $\frac{af \times w}{2ab} =$ the pressure of the lever.

6. Weigh out twenty dry Broad Beans and drop them into a graduated cylinder of cold water to measure their volume. Take them out and plant them in moist Coconut fibre or sawdust for two days. Wipe them dry quickly on the surface, weigh them and measure their volume as before.

The change in weight gives the weight of water imbibed and the volume change represents the volume of water imbibed. Do the two quantities agree ? Calculate the percentage weight of water imbibed in terms of the original dry weight. It is often over 100 per cent.

G. ADSORPTION

The marked power possessed by colloids of removing substances from solution, by attraction to the surface of the colloidal particles, is called **adsorption** and may be demonstrated by means of dyes.

The influence of electrical charge on adsorption may be well seen in the case of dyestuffs adsorbed on filter paper. Dyestuffs all contain a " chromophore " group in their molecules, to which their particular colour is due. If this group is positively charged, *i.e.*, corresponds to a kation, the dye is called *basic*, and if it is negatively charged (anion) the dye is termed *acidic*. The paper colloids in contact with water carry a negative charge. A negatively charged substance in solution, therefore, such as an acidic dye, will diffuse through the substance of the paper along with the solvent, while a positively charged substance will be fixed adsorptively at the point of contact. A mixture of two such substances will therefore be separated diffusively from each other. The phenomenon is sometimes known as **chromatography,** and it has numerous applications. See also Section 4 *G*, page 339.

The extent to which even an acidic dye will diffuse in the paper varies considerably, however, with its molecular weight and its colloidal character. In general the diffusibility runs parallel to the power of the dye to permeate living cells. Thus Light Green or Orange G will diffuse as rapidly and as far as the water in which they are dissolved, while Congo Red or Trypan Blue, for example, diffuse less than half as far, owing to their more colloidal character.

1. The differential staining action of certain well-known pairs of stains may be illustrated by this method, for example, Safranin and Light Green. Other pairs, such as Safranin and Soluble Blue or Gentian Violet and Erythrosin, will also show good results.

Take a Petri dish and cover the bottom with a wet filter paper. On this lay two short lengths of glass rod and on these a dry filter paper, which must not touch the wet one below. Make a mixture of equal quantities of 0·1 per cent. solutions of Safranin and Light Green in water. The mixture is grey or black. With a fine pipette place one drop of the mixture in the centre of the dry filter paper and shut the dish up for ten minutes. The Light Green will form a broad zone of pure green around a central red spot. This " chromatogram " may be further developed by adding carefully another drop of pure water at the centre, which will clear the red area of any residue of green and give a sharper differentiation.

2. A quantitative estimate of adsorption may be made by adsorbing Acetic acid on charcoal. The charcoal must be gently heated for a few minutes beforehand to remove air and moisture. The special charcoal for adsorption which is sold by the chemical manufacturers is the best for the purpose, as it is acid washed and does not contain too much mineral matter.

Shake up a 2N solution of Acetic acid with the charcoal and filter. Titrate with Sodium hydroxide before and after filtration.

The results should agree with the equation $\frac{C_2{}^n}{C_1} = K$, where C_1 is the amount of acid in a given quantity of the solution after filtration and C_2 is the amount of acid held by the charcoal, got by subtraction. The exponent n is usually 2, and K is a constant.

Alkaline halides such as common salt show negative adsorption, that is to say, the charcoal takes up water and concentrates the solution. This may be shown by shaking a decinormal salt solution with charcoal for a few minutes and titrating with decinormal Silver nitrate. The reaction has no temperature coefficient.

3. Enzymes are, as a rule, strongly absorbed by solids.

Shake up with blood charcoal a few cubic centimetres of Rennet, the casein-forming enzyme from the calf's stomach, and decant or filter off the clear liquid. Add equal amounts of the original and of the treated liquids to two test-tubes each containing a few cubic centimetres of milk warmed to about 40° C. Place both in an incubator at 37° C. for an hour. Note the difference in the effect in each case.

4. Make up a little Methylene Blue solution in a test-tube and add about a teaspoonful of blood charcoal. Shake up. The charcoal will adsorb the dye and decolorize the solution. Filter off the charcoal and suspend it in 100 c.c. of water. The dye is not released. Now add about 10 c.c.

of Benzene, and shake thoroughly. The dye now appears in the water. Methylene Blue is not itself soluble in Benzene (try this for yourself), but is displaced by it on the adsorbing surface of the charcoal, *i.e.*, is said to be eluted.

5. Dissolve about 3 per cent. of Saponin in a mixture of equal parts of fresh egg-white (albumen) and water. Using this mixture, blow a bubble on the end of a thistle funnel. Keep the narrow end of the funnel closed for a few moments, then open it and allow the bubble to contract under its own surface tension. The bubble shrivels as it shrinks in size and the surface is found to be semi-solid. Albumen adsorbed into a surface becomes gelated irreversibly. Compare the skin which forms on heated milk and the tough membrane formed on the outside of naked protoplasmic masses.

H. SURFACE TENSION

1. *Physical reality of the surface film.* Clean a needle with absolute Alcohol and dry it carefully. It may then be floated on the *clean* surface of water in a *clean* dish. Anything which lowers the surface tension will prevent this. Try the effect of an oil film on the water by rubbing the end of a glass rod on your nose and then dipping into the water. Remember that oils are normal constituents of protoplasm. Some substances, *e.g.*, Hexyl Resorcinol, have so powerful an effect in lowering the surface tension that they will even prevent a duckling from floating.

2. Bend a piece of stout wire into a rectangular frame and tie across it, about midway, a loose piece of cotton thread. Dip the frame flat-ways into a soap solution so as to fill it with a soap film. The tension on both sides of the thread being equal it will still appear loose. Now break the film on one side of the thread. The surface tension of the remaining soap film at once makes itself evident by shrinking as far as possible and drawing the thread into a taut curve.

3. Take a small amount of Benzene in a test-tube and colour it by adding Carmine or powdered charcoal. Add shreds of Camphor to make a saturated solution. Place a drop on the surface of *clean* water in a shallow dish. The first drop generally disintegrates, but subsequent drops will remain and will show amoeboid movements.

These are due to local differences of tension set up by differences in the rate of diffusion of the Camphor into the water at different points on the surface of the drop.

4. Place a large drop of pure Chloroform under clean water in a shallow glass dish. Draw out some fine glass thread and dip a piece about 5 mm. long into shellac solution. Dip into water to harden the shellac. Now introduce this to the Chloroform drop. It will be drawn in, as the interfacial tension between Chloroform and shellac is low, *i.e.*, the shellac is "wetted" by the Chloroform. The interfacial tension of Chloroform to glass is, however, high, so that when the shellac has been dissolved by the Chloroform the glass thread may be ejected.

Fine drops of shellac alone will also be drawn in and will curl up inside the drop, like portions of an ingested algal filament in an Amoeba.

Compare the above with the vital processes, " ingestion," " digestion," and " excretion."

5. Dissolve small quantities of (*a*) Sodium chloride, (*b*) Saponin and (*c*) Sodium cholate (Bile Salt) in water in test-tubes. Shake vigorously and observe the time taken for the foam produced to disappear. The " foam time " is roughly in inverse proportion to the surface tension, and is a useful semi-quantitative index of the amount of the lowering of the surface tension of a solvent by a solute.

6. Surface tension may be measured fairly accurately with the home-made apparatus shown in Fig. 64. A Platinum wire loop is hung from one end of a light lever, counterbalanced by means of a loop of fine chain.

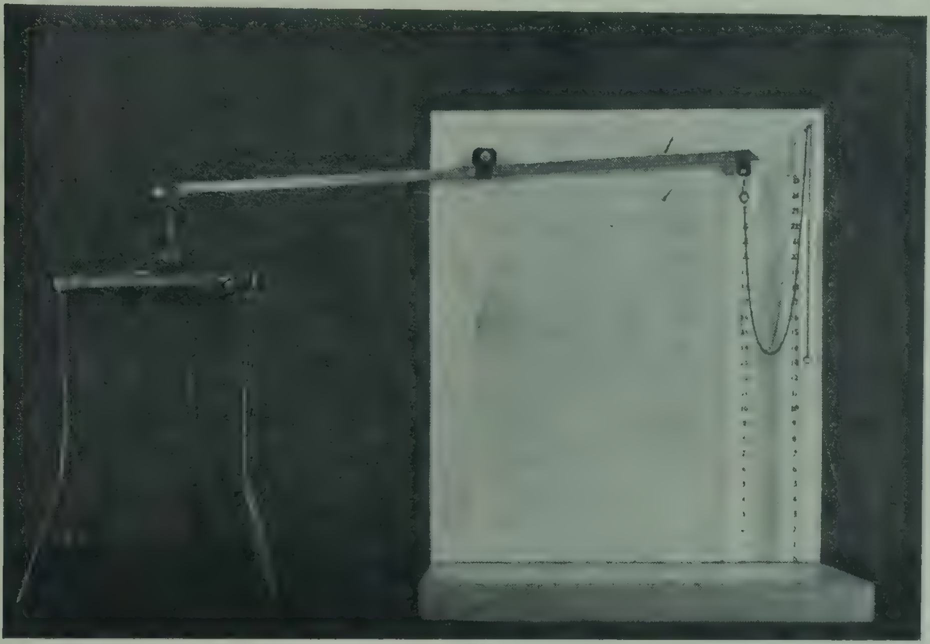

FIG. 64.—Apparatus to measure surface tension. . (*After F. E. Lloyd.*)

Clean the platinum wire loop in a Bunsen flame before each test and avoid touching it with your fingers.

Pull up the free end of the chain until the lever is horizontal. Read off the level of the bottom of the chain loop. Place the liquid to be measured in a watch-glass supported on a retort clamp and raise it till it just touches the platinum loop. Lower the chain until the loop just breaks away from the liquid surface. Repeat at least thrice and take the average reading of the bottom of the chain loop. The difference in heights represents the length of chain added to balance the tension of the liquid surface.

$$\text{Surface tension} = \frac{\text{Weight of chain added} \times 981}{\text{Circumference of wire ring} \times 2} \text{ dynes.}$$

Use Distilled water, Cane sugar, Hexyl Resorcinol, Ethyl Alcohol.

J. DIALYSIS

The separation of colloids and crystalloids by means of their different capacities for diffusion through a membrane can be easily shown in the case of a sugar and starch.

Make up a 15 per cent. solution of Glucose in water and add to it an equal quantity of a sol of " soluble starch " which emulsifies easily in cold water. Make a small sac of parchment paper (grease-proof paper), or a collodion sac prepared as below, and fill it with the mixed solution. Suspend it in a jar of water for some hours. Test the water at the end of the period with (*a*) Iodine and (*b*) Fehling's Solution. The sugar alone has passed through the membrane.

A similar result may be obtained by using mixed dyes, *e.g.*, Congo Red (colloidal) and Soluble Blue (crystalloidal).

As a perfect parchment sac is difficult to make, the following may be found useful for this and other dialysis experiments.

Make up an 8 per cent. solution of Celloidin or Photoxylin in equal parts of Ether and absolute Alcohol. Pour a few cubic centimetres into a clean dry test-tube and immediately pour out, revolving the tube meanwhile so as to cover the inside with the solution. Stand upside down to drain for a few minutes, then blow gently into the tube to dry the celloidin. As soon as the membrane formed in the tube can be detached from the glass at the lip of the tube, pull it gently away and pour water down between the glass and the membrane. The latter will then separate easily from the tube and may be pulled out as a delicate sac. Dry overnight at room temperature, then soak for twenty-four hours in 90 per cent. Alcohol (the exact strength is important). Wash in water and store in distilled water. A membrane prepared in this way is permeable to Glucose, but impermeable to Congo Red.

Section 2. Absorption

A. OSMOSIS THROUGH A SEMI-PERMEABLE MEMBRANE

1. Use a thistle funnel with a long stem and cover the expanded end with a membrane. Suitable membranes are parchment paper, or pig's bladder washed and scraped. A celloidin membrane, prepared as given above, but soaked in 70 per cent. instead of 90 per cent. Alcohol, is perhaps the best. Any of these may be fastened over the edge of the funnel with waxed thread fastened as tightly as possible. The edges of the membrane should then be trimmed with a razor blade as close to the thread as possible and given a coat of rubber solution or other waterproof cement to seal them (Fig. 65).

Alternatively, a Gelatine membrane may be tried. Dissolve 10 gm. of gelatine in 50 c.c. of warm water. When dissolved add about 0·1 gm. of Potassium dichromate and stir until dissolved. Dip the end of the thistle funnel into the Gelatine so as to form a membrane across the opening. Dry in the *light.* Wash out the dichromate by soaking in water. The Gelatine has been rendered insoluble.

Fill the funnel with 0·21 per cent.[1] Potassium ferrocyanide and place it standing in a beaker of 0·25 per cent. Copper sulphate. A membrane of Copper ferrocyanide will form in the Gelatine. After some hours (overnight) refill the funnel with 0·1M Sucrose (3·42 per cent.) containing 0·031 per cent. of Potassium ferrocyanide, and stand it in a beaker of water containing just enough Copper sulphate (0·084 per cent.) to balance osmotically the ferrocyanide inside the funnel. This is simply a precaution to keep the membrane whole.

The osmotic rise of pressure in the funnel is shown by the rise of water in the stem. With a good membrane this may soon reach the top. Provision should then be made for attaching extra lengths of glass tubing to allow for a higher column of water, or for the attachment of a Mercury manometer, in the form of a U-tube, to measure the pressure developed, which may be more than one atmosphere, *i.e.*, 33 ft. of water, an inconvenient height to measure directly.

At the end of the experiment, test some of the water in the beaker for Sucrose, by adding a little Hydrochloric acid, boiling to hydrolyse the sugar, neutralizing and then testing with Fehling's Solution (see p. 49). The membrane will probably not be perfectly semi-permeable and some sugar will have leaked out.

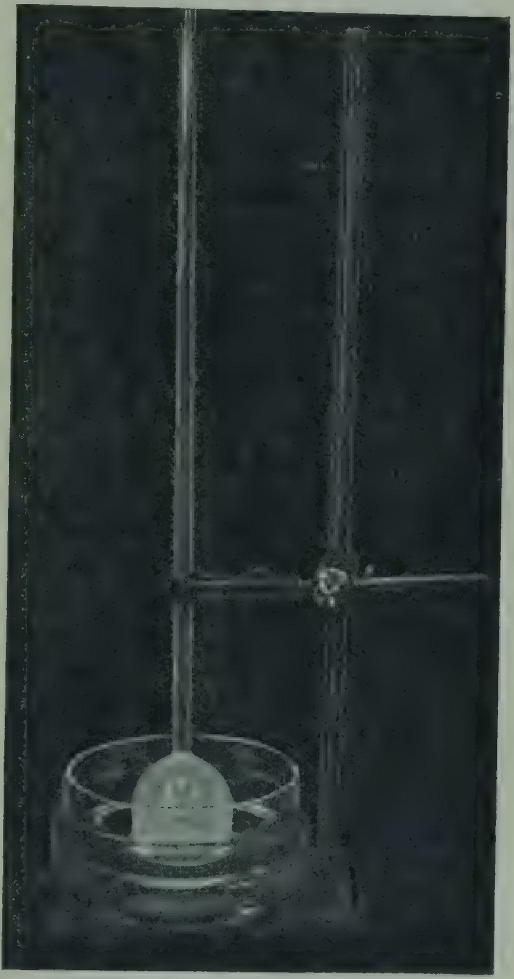

FIG. 65.—Apparatus to demonstrate osmotic pressure.

The success of the experiment depends naturally on the perfection of the membrane and of the materials easily available, collodion or celloidin are the most satisfactory. The preparation of a good collodion membrane, as suggested, involves a certain amount of trouble, but a simple one may be made by tying a piece of hard filter paper over the funnel mouth in the way described, and then soaking it in Acetic acid-collodion. This is collodion which has been damped with glacial Acetic acid and allowed to swell, and then dissolved by adding sufficient acid to make a 5 per cent. solution. Such a membrane is not strictly semi-permeable, but it is only penetrated slowly by sugar molecules and therefore will show a considerable, though temporary, rise of water in the funnel.

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[1] By pouring from a pipette down a thin wire.

Instead of sugar one may use Diethylene glycol. The advantage lies in the relatively greater concentrations which may be used. For example, a 50 per cent. solution of Diethylene glycol is approximately of 10 molar strength, a concentration which could not be obtained with a sugar. With such a concentration and a simple parchment paper membrane a rise of water of 3 or 4 metres may be readily obtained.

2. Dissolve 2 per cent. of Agar in water and pour it carefully down a glass rod into a U-tube, so that it sets to form a short plug in the curved part of the tube. When it has set, pour into one limb of the tube a 2 per cent. solution of Potassium ferrocyanide and into the other a 10 per cent. solution of Copper sulphate. The latter is hypertonic to the former, *i.e.*, it has a higher osmotic pressure.

The Agar is, in effect, a permeable membrane, but a semi-permeable membrane of Copper ferrocyanide forms within it where the two salts meet in the course of diffusion. Leave the tube some time for observation, marking the levels reached by the fluids in each limb of the tube. Watch also the position of the deposited membrane.

B. ARTIFICIAL OSMOTIC CELLS (Traube's Cells)

1. Grind up together in a mortar equal parts of Copper sulphate and Sucrose. Moisten the powder slightly and mould it into pills about the size of small peas. Set aside to dry. Make up a 2 per cent. solution of Potassium ferrocyanide in a 5 per cent. Gelatine suspension and place about 300 c.c. in a tall beaker. Drop in one of the " blue pills " and leave standing in a warm place where it will be quite undisturbed. The membrane of Copper ferrocyanide which forms around the pill allows the entry of water, but prevents the diffusion of the sugar. A growing cell results, which in the course of time will send out processes which will branch and reach the top of the fluid. The purpose of the Gelatine is simply mechanical, to support the growth against the effects of vibration.

2. Dissolve 1·5 gm. of Sucrose in 10 c.c. of 2 per cent. Tannic-acid solution. With a pipette introduce a large drop of the mixture into a beaker containing 200 to 300 c.c. of a 5 per cent. Gelatine solution. In this case the semi-permeable membrane formed is of Gelatine tannate. Growth follows, as in the first example.

C. POTATO OSMOSCOPE

The working of osmosis through a tissue of living cells may be shown very easily with a tuber or a thickened root.

Cut both ends off a large Potato and hollow out a portion in one end, into which a little solid Sucrose is introduced. Stand the other end in a small quantity of water. In a short time the water will be absorbed and the hollow where the sugar is will be overflowing with liquid.

A large Carrot may also be used. In this case make a clean hole with a cork borer, about $\frac{1}{2}$ in. across and 1 in. deep, at the stem-end of the Carrot. Place some sugar in the hole, fill it up with a strong sugar solution and insert in the hole a rubber stopper carrying a glass tube. It may be necessary to bind the stopper down with tape. Slice the tip off the Carrot and immerse the whole in water. The rise of water in the glass tube gives a direct measure of the rate of absorption.

D. OSMOTIC POTENTIAL OF A SOLUTION (Barger's Method)

The Osmotic Potential or Osmotic Pressure of a solution of unknown concentration (*e.g.*, a tissue extract) can be simply measured by comparing its vapour tension with that of a standard solution. This can be done with very small quantities of solution enclosed in capillary tubes.

Draw out a number of glass capillary tubes about 1·0 mm. in diameter and 3 in. long. Prepare a few cubic centimetres of the following molar dilutions of Potassium nitrate, 1·0, 0·7, 0·5, 0·2, 0·1, 0·05. Dip the end of the capillary into the 1·0M solution and allow a drop about 5 mm. long to enter the tube. Tilt the tube so as to run the drop a little way up the tube and then draw up a similar drop of the unknown, separated from the first drop by a small air space. Repeat this with alternate drops of standard solution and of the unknown solution until about half a dozen drops in all have been taken up. Touch the two ends of the capillary with hot wax to seal them up. The whole procedure only takes a few minutes. Repeat the process with five other capillaries, using different dilutions of the standard in each capillary. Now arrange the tubes in order of concentration and stick them down with wax or plasticine side by side on a microscope slide.

Place under the microscope and with a low power objective and an eye-piece scale, or with the aid of a mechanical stage, measure carefully the length of one or two drops of both solutions in each tube. Maintain at a constant temperature for an hour. It is usually sufficient to keep the slides in a flat trough of water which can be placed on the microscope stage to prevent rapid changes of temperature during measurement.

Re-measure the original drops. Those drops which have a higher osmotic pressure than their neighbours (*i.e.*, have a lower vapour pressure) will have increased in length, and vice versa. These observations tell us which concentrations of Potassium nitrate are stronger than the unknown and which are weaker.

A second set can now be put up using closer steps in concentration of the standard, over a narrow range which includes the presumed balance point. This second set should show, with close approximation, which strength of Potassium nitrate is in equilibrium with the unknown, and as the osmotic pressures of Potassium nitrate solutions are known (see Appendix, p. 464) the osmotic pressure of the unknown can be calculated.

By plotting on graph paper the *percentage* increase of drops of stronger solution and the percentage decrease of drops of weaker solutions, the zero point of balance can be found with fair accuracy by intercalation.

Plot the concentrations of the standard used as abscissae and the percentage change of length of the drops as ordinates with the zero in the middle of the scale. The percentage change of length of drops of the unknown when balanced against the various standards are then plotted. Increased lengths go above the zero line and decreased lengths below. Join up the points and drop a perpendicular, from the intersection of this line with the zero line, to the scale of concentrations. This will indicate the concentration with which the unknown should be in equilibrium and the result *should be checked by trial.*

E. OSMOTIC PRESSURE IN LIVING CELLS

Rhubarb. – Cut thin epidermal sections from the red-coloured petiole of Rhubarb and place them in water in a watch-glass. Mount sections in Sucrose solutions varying between 0·25 and 0·05M and examine under the microscope. Potassium nitrate in concentrations between 0·2 and 0·1 molar will be found to work well instead of sugar. A solution of Potassium nitrate of 0·13M strength has the same osmotic value as a Sucrose solution of 0·21M. These concentrations will be found to be about right for many cells.

Sketch the appearance of cells in different sugar concentrations and work down, by making intermediate concentrations, to that which produces only a slight trace of plasmolysis, as shown by the withdrawal of the coloured cell contents from the wall at the corners only. This is the **isotonic concentration.**

The **isotonic coefficient** of a substance is the ratio between the concentrations of a solution of cane sugar and a solution of the substance which are isotonic with one another, or with a third solution, *e.g.*, a cell sap. It is denoted by i, and is calculable as follows :—

$$i = 1 + (n - 1)\, a$$

where n = the number of ions into which the salt dissociates in solution (*e.g.*, KNO_3 = 2) and a = the dissociation constant or percentage of dissociation at the given concentration.

Given that the dissociation constant of 0·1M KCl is 0·8 (80 per cent.), calculate its isotonic coefficient and make up and test a solution which should be isotonic with the Rhubarb petiole cells which were previously tested with sugar solution.

F. SUCTION FORCE

Suction force has been described as the force per unit area with which water begins to enter the cell when it is immersed in pure water. Colloquially speaking, it represents the " thirst " of the cell.

The principle of the method of estimation is that the cell draws in water with a force the magnitude of which is determined by the difference (if any) between the osmotic pressure of its sap and the counteracting elastic pressure due to the distended protoplasm and cell wall. This suction force can be measured by balancing it against the osmotic pressure of solutions of various strengths. In a solution of which the osmotic pressure equals the suction force there will be neither gain nor loss in weight by the cells.

It should be understood that if tissues are placed in water before examination they will become saturated, that is, their suction force will be zero. They will then only be in equilibrium with water. Endeavour should, therefore, be made to use tissues which are as nearly as possible in their actual living condition in the plant.

1. *Taraxacum.*—Make up about 300 c.c. of a solution of Sucrose of 0·25M strength (M=342). From this make dilutions to the following strengths: 0·20, 0·18, 0·15, 0·10, 0·05, 0·01M. Take the flower stalk from a Dandelion or some other similar stalk, *e.g.*, Daffodil. Cut it into three or four short lengths and split each length into four strips. Put them in a corked tube to keep them moist. Fill six shallow dishes with the sugar solutions and place a strip of the flower stalk into each. Note the resulting curvature. If this is outwards the sugar solution is hypotonic to the living cells, *i.e.*, they absorb water from it and expand. If the curve is inwards, on the contrary, the solution is hypertonic. The pith cells then lose water and shrink. Note the two concentrations between which the curvature changes. Let us suppose that in the 0·10M solution the curvature is outwards, but is inwards in all the stronger solutions. Make up a series of sugar dilutions between 0·01M and 0·15M and test other strips of tissue in these. Note again the limiting concentrations between which the curvature changes occur. When a strength of sugar has been found in which the strip remains straight, or at least unaltered in shape, that concentration has an osmotic pressure which equals the suction force of the cells. The osmotic pressure of the sugar solution may be found by reference to the table in Appendix, page 462.

Sodium chloride may also be used in a series of dilutions from 0·8 to 0·2M, or Potassium nitrate in dilutions between 0·8 and 0·1M.

2. Punch out a core of Beetroot with a cork borer about ½ in. wide and cut it transversely into uniform slices as thin as possible, placing them in a closely covered dish.

Prepare Sucrose solutions of the following strengths: 0·5, 0·4, 0·3, 0·1M (M=342) which may be conveniently made by dilution from the 0·5M solution. Place about 100 c.c. of each solution in a series of beakers and add an equal number of weighed Beetroot slices to each. The weighing must be done in a closed tube to prevent loss by drying. After an hour take out each lot of slices in turn, dry the surfaces quickly with filter paper and weigh again. Note the sugar concentration in which there has been neither gain nor loss in weight. This is the solution the osmotic pressure of which balances the suction force of the cells. It may be necessary to use solutions of intermediate strength to get an exact balance.

Compare the suction force thus determined with the full osmotic pressure of the cells by the plasmolysis method as given for Rhubarb under E (see p. 306). Cut thin microscopic sections and observe them in solutions of varying concentration, noting the first signs of plasmolysis as the index of a solution which is isotonic with the cell sap.

3. *Molz' Method.*—Prepare sugar solutions as above and put small quantities into separate closed vessels, such as short corked glass tubes. Select a shallow glass dish which can be placed on the microscope and fill it with liquid Paraffin. Now cut up some soft tissue, *e.g.*, a flower stalk, into lengths of 2 to 3 mm. Immerse each in turn in the liquid Paraffin and measure its length accurately with a microscope. A measuring microscope which will measure to 0·01 mm. is naturally the best. Wipe off the Paraffin with filter paper and transfer each piece to one of the sugar solutions, keeping a note of the measurements. After an hour replace the pieces in the liquid Paraffin and re-measure. The sugar concentration which produces neither shrinkage nor elongation is that which balances the suction force.

The same type of measurement can be made yet more accurately by using only a few cells, such as small pieces clipped from a Fern prothallus. In this case a camera lucida is used to make scale drawings of selected cells lying in liquid Paraffin, and the drawings are repeated in each case after immersion in one of the sugar concentrations. There should be no change in the size of the cells in the concentration which balances the suction force.

G. TISSUE STRAINS

As the degree of turgidity differs in different tissues there are strains constantly set up between tissues in the living organism, due to their different swelling powers. The balance of these strains often contributes importantly to the mechanical rigidity of unlignified young organs.

Select a young green shoot of Elder of the current year's growth. Cut a length of 2 or 3 in., with clean squared ends and measure its length as accurately as possible. Make four shallow longitudinal slits with a razor blade and quickly peel off the outer tissue in four strips, leaving the pith free. Immediately re-measure the respective lengths of the separated tissues. What do you conclude respecting the strains in the intact stem ?

Similar measures may be made with other tissues. For example the skin of Rhubarb petiole shows a marked change when stripped off. Again a narrow ring of bark may be cut from a smooth branch of Birch or Hazel. When it has been separated try immediately to fit it back into place. It will show marked shrinkage.

H. PLASMOLYSIS. (See also in Part II, Section 1, p. 35.)

The shrinkage of the protoplasmic sac known as plasmolysis may be best seen where coloured cell contents exist. Good material is easily obtained, *e.g.*, surface sections from the underside of the leaves of *Zebrina pendula*, *Begonia rex*, *Coleus* or *Fuchsia* sp. which have purple colouring, or from the stems of *Stachys sylvatica* or the epidermis of Rhubarb petiole.

1. Mount sections in 10 per cent. Sucrose solution and sketch the appearance of the cells in the plasmolysed state. Remount the sections

in water after washing off the sugar solution and note the time taken for the restoration of full turgidity. Other solutions may be tried, *e.g.*, 3 per cent. Sodium chloride (sea-water strength) or 10 per cent. Potassium nitrate, and their effects compared.

2. Plasmolysis depends on the non-entry of the plasmolysing substance into the cell vacuole. In most cases this is really slow entry, not absolute exclusion, so that equilibrium is slowly re-established and plasmolysis disappears.

Mount sections in 10 per cent. Glycerine. Sketch the appearance of the cells, then set aside for a couple of hours. It will be found that there has been almost complete de-plasmolysis.

Repeat the above experiment, using solutions of 1, 3 and 5 per cent. How is the rate of penetration affected by the concentration of the plasmolysing substance ?

3. Plasmolysis is not always complete. Test the cells of Fern prothalli in comparison with the above. It will be found that certain portions of the utricle remain adherent to the wall throughout. Even in cases where more or less complete shrinkage occurs, fine strands may be seen connecting the shrunken protoplast to the pits in the cell wall.

Such differences are probably due to difference in the physical state (*e.g.*, viscosity) of the protoplast.

J. PENETRATION BY SOLUTES

The passage of dissolved substances into the cell is apparently governed by several factors, among which are (*a*) size of the molecule (micro-sieve effect), (*b*) solubility, especially in lipins, (*c*) the affinity of the molecule for water and (*d*) adsorption to constituents of the protoplasmic utricle.

1. An illustration of the micro-sieve effect is provided by the dialytic separation of Congo Red and Soluble Blue, see page 302. Here the size of the molecule or molecular aggregate is the controlling factor.

Penetration by solubility probably operates especially in the case of molecules undissociated in solution, which enter more readily than ions. Size of molecules is not so important in this case, but the possession of a polar group in the molecule (*e.g.*, $-\mathrm{OH}$) which has an affinity for water is decisive, the penetrating power agreeing with the solubility of the molecule in non-polar organic solvents (*i.e.*, those with symmetrical molecules), among which may be classed the lipins. Thus, the less polar the substance the more soluble it is in the lipin and the more penetrating. The majority of rapidly penetrating substances seem to enter in this way.

2. Make up dilute solutions of Water Blue and of Neutral Red in 0.05M Calcium chloride. The solutions must be very dilute, so that they are only lightly coloured (about 1 : 10,000). The function of the Calcium chloride is only to prevent staining of the cell wall ; it does not enter the cells. Mount small pieces of epidermis from the inner scales of an Onion or Hyacinth bulb in a drop of each solution. Alternatively, float rooted *Lemna* plants on the

solutions or steep in them small seedlings of Mustard, with root hairs, for some hours.

Examine under the microscope for dye penetration. What parts of the cells are stained ?

Dip fresh material of one of the above for a few moments into absolute Alcohol. Wash and test for dye penetration. What differences do you observe and why ?

3. Soak a few *Lemna* plants in a 1 per cent. solution of Ether for a few minutes and test the dye penetration in the narcotized cells of the roots. Put aside control specimens which have been treated with Ether, but not stained, for a few hours in water and then test them. Is the Ether effect permanent like the Alcohol poisoning ?

4. Test cells of Onion scale or of the coloured epidermis of Rhubarb with 20 per cent. solutions of the following substances :—

Ethyl alcohol	$C_2H_5\,OH$	One polar group.
Ethylene glycol	$C_2H_4\,(OH)_2$	Two polar groups.
Glycerol	$C_3H_5\,(OH)_3$	Three polar groups.

What would you predict regarding their penetration on the basis of their chemical constitution ? How would their differing penetration be observable ?

5. A chemical model of penetration by solution may be made as follows : Make a 3 per cent. solution of Lecithin (a phospholipin) in Xylol. Prepare a few cubic centimetres of aqueous solutions of (*a*) Neutral Red (apolar, lipoid-soluble) and of (*b*) Methylene Blue (polar, lipoid-insoluble) in two test-tubes. Float the Xylol solution on top of the dye solutions and put aside for observation. Do both dyes penetrate the Lecithin solution ?

6. Dissociated ions of salts seem to penetrate chiefly by adsorption to protoplasmic constituents. A chemical model of such penetration is provided by the following system. Methylene Blue does not penetrate by solution (see above), but it readily does so by adsorption. Shake up some Methylene Blue solution with a little blood charcoal and filter. Suspend the charcoal in liquid Paraffin. Melt some crystals of Phenol by warming and pour the liquid into the bottom of a test-tube. Carefully overlay this with about 1 cm. of water, on top of which is layered the Paraffin suspension of charcoal. Particles of charcoal will fall through the water without releasing the adsorbed dye, but give it up to the Phenol which becomes deeply coloured.

In the living cell Brownian movement may carry particles carrying an adsorbate through the utricle from the outer to the inner surface, where it is released to the vacuole.

K. CHANGES IN PERMEABILITY

The penetration of ions is considerably influenced by their own hydration and their effect on the hydration of protoplasmic substances. Ions with opposite effects in this respect will show **antagonism.** This is clearly shown between Sodium and Potassium on the one hand, which increase the hydration of protoplasm, and Calcium and Magnesium on the other, which reduce it, thus bringing the micellae closer together. Calcium will thus antagonise the entrance of Sodium, or vice versa.

1. Make up a 1·8 per cent. (0·32M) solution of Sodium chloride. Take two test-tubes and fill one with water and one with the salt solution. Drop into each a washed slice of fresh Beetroot. On standing, colour will be seen to escape from the slice in the salt, but not in the water, showing that Sodium chloride has increased the permeability of the cells. To another sample of the salt solution add 1·88 per cent. (0·17M) of Calcium chloride. Place a washed slice of Beetroot in the mixed salt solution. No colour escapes. The presence of the Ca ions antagonizes the activity of the Na ions. This is of great importance in nature, especially in the sea, since the above experiment shows that the Na ions by themselves are toxic to cells, as shown by the escape of pigment, but the presence of Ca ions makes the sea-water a physiologically " balanced " solution.

2. To illustrate the effect of temperature on permeability, one may make use of the escape of the colouring matter (anthocyanin) from the cells of the Beetroot. Cut a number of pieces of Beetroot about 2·0 cm. by 1·0 cm. by 0·25 cm., keeping them as nearly as possible the same size. Drop them into tap water and wash them thoroughly.

Fill a large beaker with water and bring to the boil. Place in it a test-tube containing 10 c.c. of distilled water and stir the water with a thermometer until it reaches 100° C. Drop in a slice of Beet and continue stirring until the water has attained a definite purple colour. Note carefully the time required for this. Pour off this solution into another test-tube, cool it under the tap and keep it as a standard. Repeat the process with water at 90° C., and then by successive downward stages of 10° to 50° C., using the same amount of water and a fresh slice of Beetroot for each determination. In each case note the time required to reach the standard colour. Plot these results on squared paper and calculate the relative permeability and the temperature coefficient for each rise of 10° C. in the series (see p. 366).

3. Mount a few long filaments of *Spirogyra* on a slide in water and heat one end of the slide *carefully* over a Bunsen burner. Examine under the microscope (low power) and notice the changed appearance of the cells near the heated end.

Add a drop of strong Eosine solution and note how the permeation of the dye into the cells is affected by the action of heat on the protoplasm.

L. WATER CULTURES

Plants may be grown to maturity in a solution of nutrient salts, without any soil,[1] provided the solution is kept well aerated. This may be accomplished simply in large glass or glazed pots (7 lb. jam jars) fitted with cork bungs, through a hole in which the stem of the plant is passed (Fig. 66). The pots must be covered to keep out light, otherwise the roots will become

[1] See Appendix, page 461, for suitable solutions.

infected with algal growths, and a glass tube must be inserted leading to the bottom of the pot, through which air is daily blown from a bellows or bicycle pump. It is best to wax the corks with paraffin wax and to support the plant, while small, by packing the hole in the cork with non-absorbent cotton wool or asbestos wool.

Several other methods of setting up water cultures have been devised; for example, growing plants in clean washed sand in wide tubes or flower pots standing in a shallow layer of culture fluid, which is drawn up to the roots by the capillary action of the sand. In such a case artificial aeration is not needed, but the culture solution should be replaced daily with fresh fluid, as it evaporates rather rapidly. The bottom of each pot should be covered with a layer of washed river gravel, over which the sand is packed in. Work up the sand with the culture fluid beforehand and pack it into the pot while damp.

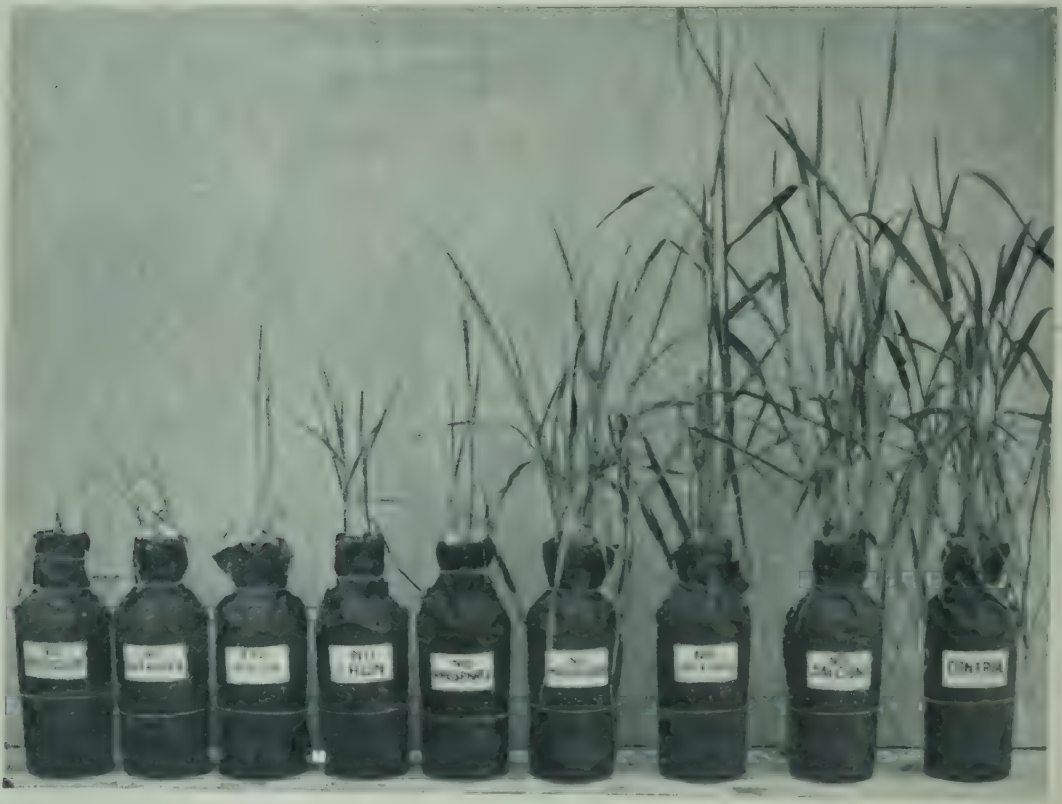

FIG. 66.—A series of water culture experiments.

Another "set-up" is called the sheet method. A plate of glass, about a foot square, is supported in a wooden holder at an angle of 15° with the vertical. Cut a sheet of white blotting paper a foot wide and about 18 in. long, wet it and apply it to the upper side of the glass, the extra length being turned over so as to dip into a pan of the culture solution. The paper is thus kept wetted by downward capillarity. A germinated seed is placed between paper and glass near the upper edge. Its roots will develop extensively in two dimensions, and the whole growth is visible through the glass and easily preserved, if desired, by pressing between drying papers after the experiment. Here, again, it is desirable to exclude light from the roots. Black blotting paper may be used if obtainable.

Perhaps the neatest method is that of using gemmae of *Marchantia* as material, planting them on Agar in Petri dishes (see p. 80). The Agar is made up at 4 per cent. strength in water and then diluted with an equal volume of the culture fluid before pouring into the plates to set solid.

For the previous methods many plants may be used as subjects, the common annual Sunflower being as good as any. Wallflowers, Cress and Buckwheat are also very suitable. Cereals may also answer, but they are rather liable to mildew disease when grown indoors. Plants with very large seeds should be avoided.

The seeds should be germinated on wet blotting paper or sand in a glass dish, and the seedlings set up in the culture solutions as soon as they are large enough to handle.

The great advantage of the water culture method from the physiological standpoint is that one can learn, by omitting a particular element from the plant's food, what effect that element has on metabolism. For this purpose standard variations of a complete nutrient solution have been worked out, and will be found in Appendix, page 461.

M. ABSORPTION OF WATER BY THE SEED

1. To test the entry of water into the seed during germination get a cork bung, or cut a piece of sheet cork into a 3 in. disc. Notch the edge so as to hold about a dozen Broad Bean seeds, pushed well into the notches. Fix half the seeds with the scar (hilum) downwards and half with the scar upwards and float the whole on water. Compare after a day, as regards the amount of swelling. If possible, it is best to weigh each lot of seeds separately before and afterwards.

2. Take another dozen seeds of Broad Bean and carefully cover the micropyle in half of them with rubber solution (tyre-mending cement). Allow it to dry and then weigh each lot of seeds. Place them in a large pan of water. Take them out at about twelve-hour intervals, wipe them dry on the outside and re-weigh. The untreated seeds will absorb water to about 120 per cent. of their dry weight before reaching their maximum, but the sealed seeds may not reach half that weight in the same time.

Section 3. Transpiration

A. THE POTOMETER. (Combine, if possible, with the Porometer on p. 321. See also under G, p. 322.)

The potometer is designed to measure the amount of water absorbed by a living plant, but not the amount given off (see next experiment). This is done by observing the rate of movement of an indicator bubble in a fine bore tube, which is a sensitive gauge of the rate of water intake.

1. The standard form of potometer is that devised by Francis Darwin

(see Fig. 67). It consists of a short straight tube about 1 cm. wide with an L-shaped branch sealed in at one side. The plant (a leafy shoot of any woody plant will serve [1]) is inserted at the top of the branch tube and sealed in, either through a hole in a rubber cork or with a joint of stout rubber tubing. The former is better and the stem is easily inserted by pushing a cork borer, one size larger than the hole, through the hole in the cork so as to widen it, then pushing in the plant stem at the cutting end of the cork borer, and lastly withdrawing the borer so as to leave the stem gripped in the cork. The upper end of the straight tube is closed by an unbored rubber cork and the lower end by another rubber cork, through which passes the end of a piece of 1 mm. bore glass tube about 12 in. long.

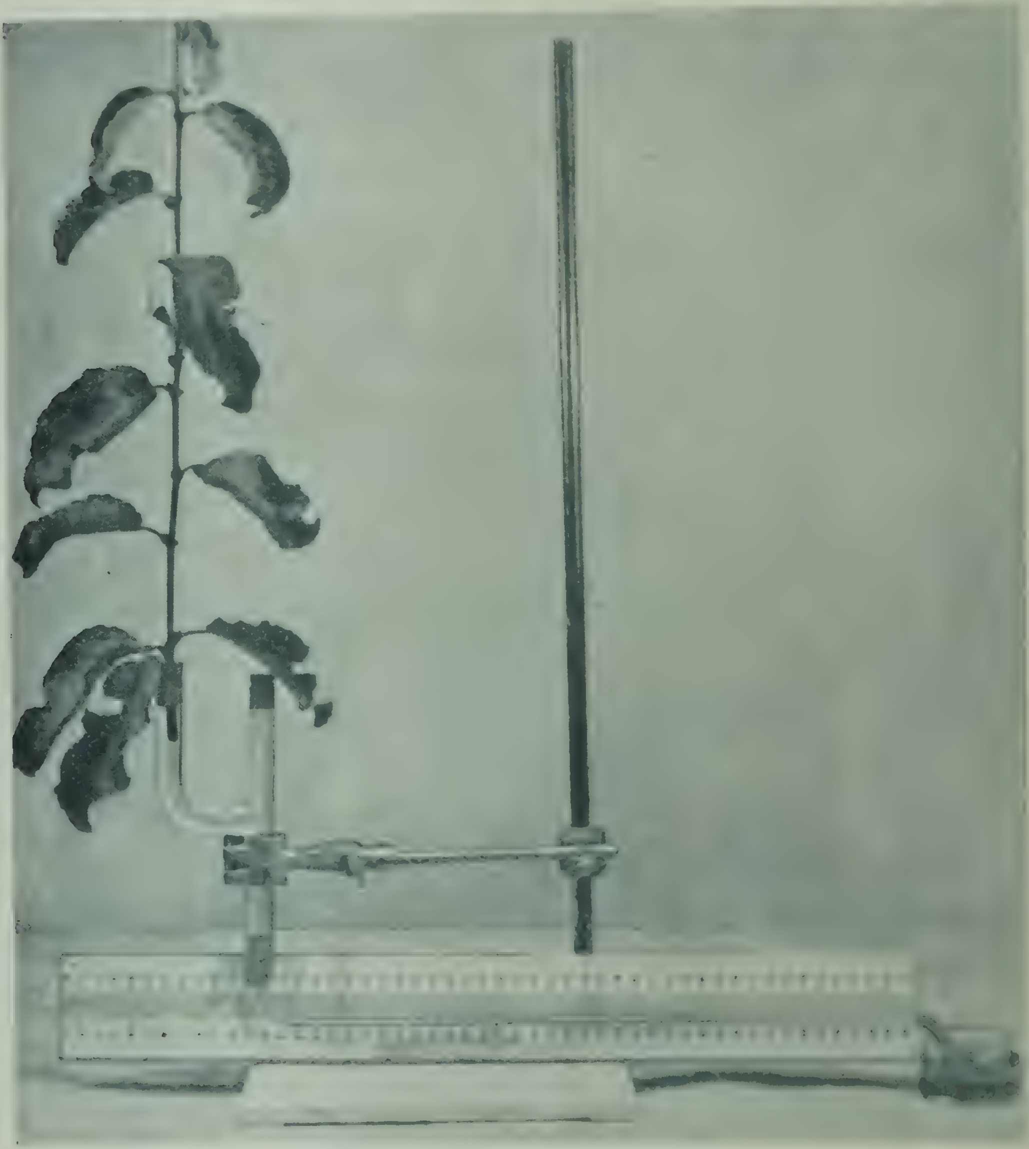

Fig. 67.—The Darwin potometer.

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[1] Branches for use in the potometer should be cut from the tree under water by bending a twig under water in a basin and cutting it with sharp clippers. This prevents the entry of air into the vessels. The branches may then be kept in water until required.

In winter, branches of Common Yew will be found to work very well.

Place the finger on the lower end of this fine tube and fill the whole apparatus with water. Insert the cork and the plant into the upper end of the straight and the side tubes respectively, taking care to see that *no air bubbles* are trapped below the plant. Lower the end of the fine tube below the surface of water in a small dish, remove the finger and secure the apparatus in a clamp. Allow it to stand for a short period, then withdraw the water dish and allow an air bubble to enter the narrow tube. Mark the tube in 5 cm. lengths and time the rate of movement of the bubble, which eventually escapes to the top of the straight part of the apparatus. If the bore of the tube is measured its volume can be calculated, and so the rate of water uptake approximately ascertained.

The effect of different circumstances may now be tested :—

(*a*) *Temperature.*—Move the apparatus to different parts of the room, noting the relationship of temperature to absorption.
(*b*) *Humidity.*—Spray water on the upper side of the leaves and hang a wet sheet round the plant.
(*c*) *Wind.*—Place the apparatus in the draught between an open door and window.
(*d*) *Evaporating Power of the Air* (see Section 3 D, p. 319).—Plot the ratio between transpiration and evaporation (T/E).

These varying conditions really affect the amount of water lost by evaporation from the leaves, and the effect on the water absorption, as measured by the potometer, shows that there is a certain correspondence between these quantities. For further information on this matter we must turn to the next set of experiments, page 316.

To compare one plant with another in the potometer it is necessary to calculate the leaf area of the specimen used and to reduce the measurements of absorption to a standard, namely cubic centimetres per square decimetre of leaf surface per hour. In some cases this is simple, *e.g.*, in *Taxus* (Yew), in which the leaves are so uniform that it is only necessary to measure the area of one leaf and multiply by the total number. In most plants, however, it will be necessary to measure each individual leaf. If the leaves are evenly lanceolate it will be sufficient to take the area as equal to two-thirds of the length multiplied by the maximum breadth, but if the leaves are irregular in outline, resort must be made to tracing each leaf on squared paper and either counting the number of squares covered or cutting out and weighing the outline in comparison with a known area of the same squared paper. The ratio of the two areas is then assumed to be the same as the ratio of the two weights.

Compare a deciduous and an evergreen plant in the potometer under similar conditions. Cut epidermal sections of the leaves of each type and count the number of stomata in each per unit area, *e.g.*, the field of the high power of the microscope.

2. Other types of potometer are used, one of which, of sturdy construction, is shown in Fig. 68. This is known as Thoday's pattern. Another commonly

used is Farmer's pattern, in which the shoot is inserted through a cork into a bottle, together with a horizontal capillary tube and a tapped funnel to replenish the water in the bottle. This potometer, however, possesses one serious objection. Owing to the large volume of water needed to fill the container the instrument can very easily operate as a water thermometer if sunlight is allowed to fall on the bottle. If care is not taken therefore, the bubble can be observed travelling in the wrong direction, due simply to expansion of the water. In each of the above patterns the capillary tube is horizontal, which is supposed to give greater sensitiveness, and one bubble only is used, which is returned to the starting-point after each reading by letting more water into the system from the funnel. The latter pattern with the bottle has the advantage that rooted plants may be used as well as cut shoots. As it is easily constructed the student is advised to make one and to test the rate of absorption in a small plant, say of *Fuchsia* or *Pelargonium*, first with, and secondly without, roots. It is interesting to see whether, under the circumstances, the presence of roots actually promotes or hinders the absorption of water by the stem. Note what results you get. What explanation can you suggest for them?

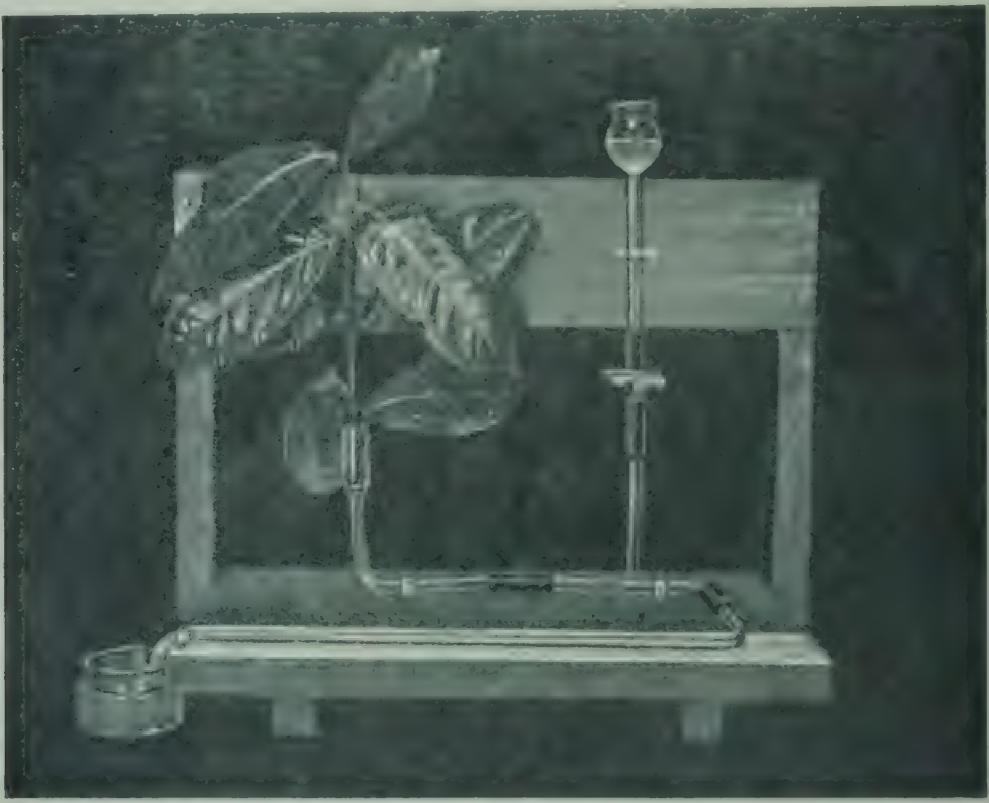

FIG. 68.—The Thoday potometer.

B. COMPARISON OF ABSORPTION AND TRANSPIRATION

For this purpose it is necessary to measure the water lost by evaporation from the leaves as well as the water taken up by the roots or by the cut stem during the same time. The former factor is usually measured by weighing.

1. If the experiment is not to last for more than a few hours the simplest arrangement is to connect the shoot by thick rubber tubing to the bottom of an untapped burette, *i.e.*, a burette intended to work with a rubber tube and spring clip instead of a tap. Suspend the burette with the shoot hanging downwards and pour in water so as to half-fill the burette, then add a few drops of oil to prevent evaporation. If there is any leakage of water, wire the rubber tubing closely to both plant and burette. A cheap, hanging, druggist's balance may be used for weighing, or one may be improvised, with a light wooden or metal rod about 2 ft. long as a beam, suspended either from a loop of silk or preferably by a bent tin stirrup resting on a piece of glass rod or on the edge of a penknife. A tin lid, hanging from three

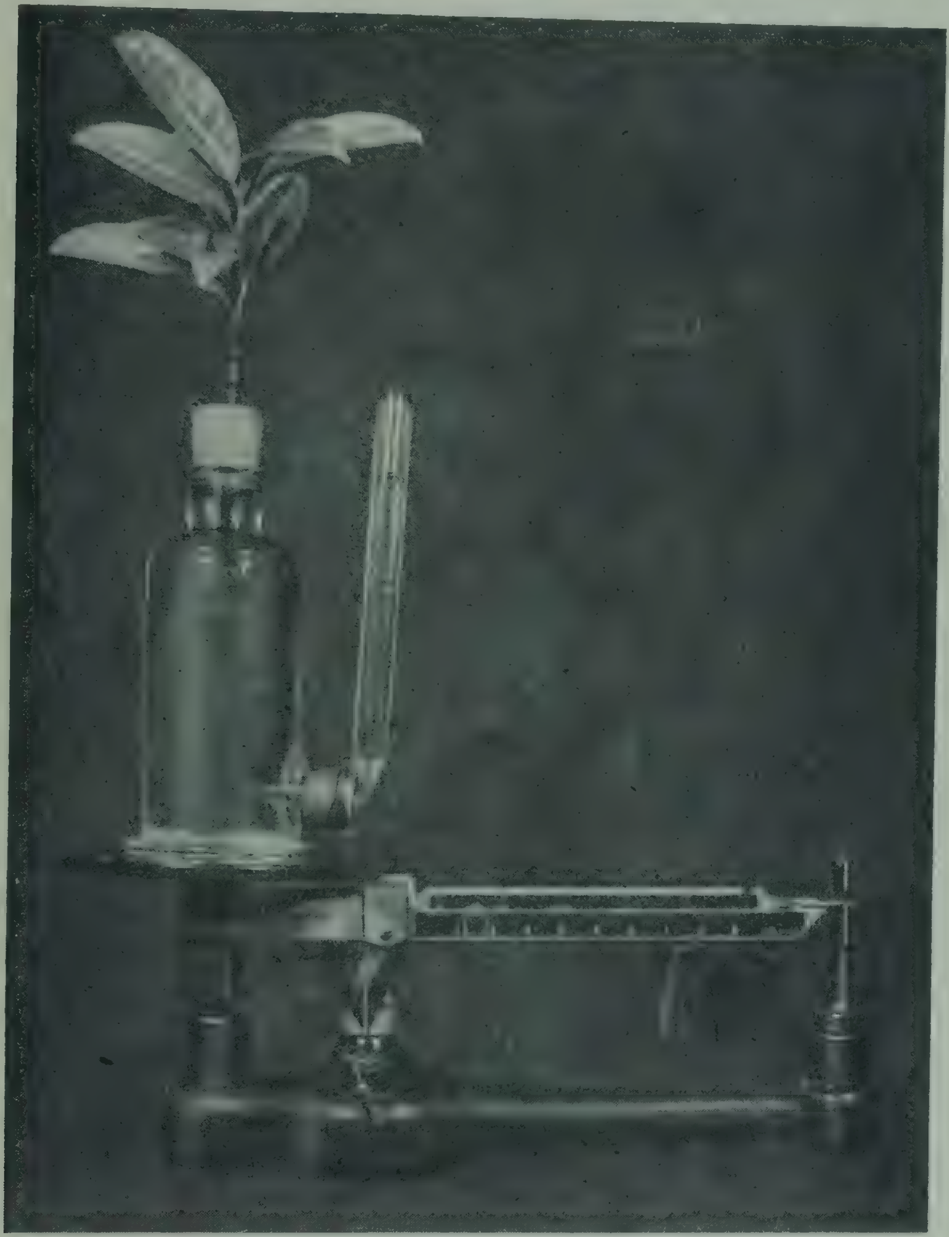

FIG. 69.—Apparatus to compare absorption and transpiration.

threads, may be attached at one end for weights and the burette hung from the other end. A balance of this kind may be made sensitive to 0·1 gm. The amount of water lost is measured by the loss in weight and that absorbed is read off on the burette.

2. For experiments lasting over several days it is best to use an apparatus such as that in Fig. 69. Here the plant (which may be rooted or not) stands in water in a bottle large enough to provide a good reservoir. A short burette tube with a horizontal neck is attached through a corked opening at the side of the bottle. A few drops of oil should again be placed on the water in the burette to check evaporation. The whole apparatus may stand on the pan of an open-pan balance and be weighed at frequent intervals.

If there is any difference between the quantity of water absorbed and the quantity lost, what becomes of the difference? Can you suggest ways in which it may be utilized by the plant?

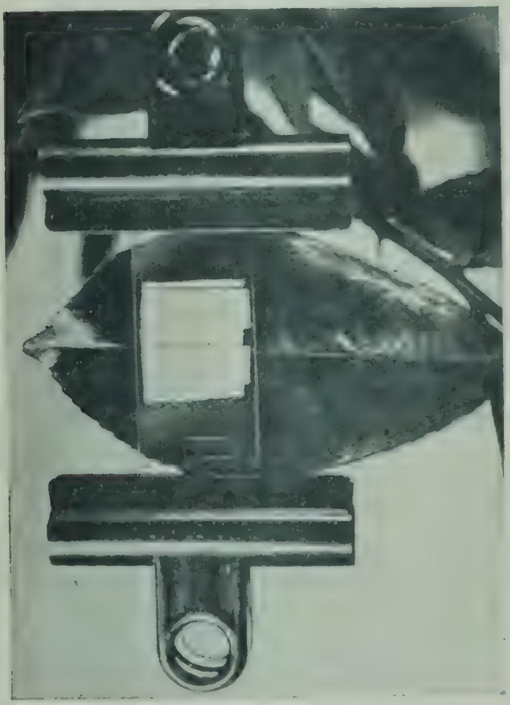

Fig. 70.—Method of determining transpiration using Cobalt chloride paper.

C. COBALT CHLORIDE TEST FOR TRANSPIRATION. (See also Part IX, p. 427.)

This is a chemical means for demonstrating the evaporation of water from the leaf surface. By standardizing the test papers against a standard evaporating surface, say at 1 mm. distance from wet filter paper, it may be converted into a semi-quantitative method for comparing transpiration rates of plants in the field.

1. Soak filter paper in a 5 per cent. solution of Cobalt chloride in water and hang it up to dry. Cut it into strips about 1 in. by $\frac{1}{2}$ in. and dry these thoroughly in an oven. The original colour of the wet paper is pale pink, but when dried the colour becomes an intense blue (Cobalt Blue). The dry paper is thus a moisture detector, turning pink when left in the air or when placed near an evaporating surface. The time taken for the blue colour to disappear is a measure of the amount of evaporation from the moist surface.

The paper strips should be stored in a well-corked tube, over Calcium chloride, and may then be preserved or carried into the field.

Select a suitable leaf attached to a growing plant, lay a piece of Cobalt paper loosely on the surface and immediately press it gently down between two pieces of glass, held one above and the other below the leaf (see Fig. 70). Get an assistant to start timing from the moment the glasses are pressed together till the last vestige of the blue has gone.

Compare the respective rates of loss from both upper and lower surfaces of the leaf, from young and old leaves, and from leaves of different types of plant. Test also plants growing in different situations, sunny and shady, or exposed and sheltered, in the field. The method is widely applicable and yields valuable ecological data.

2. Test two plants of Broad Bean, one of which has been in the dark for some hours. If its stomata are closed there should be a marked reduction in the water loss from the leaf surface. Note that the Broad Bean has stomata on both leaf surfaces.

D. MEASUREMENT OF THE EVAPORATING POWER OF AIR.

(See also Part IX, p. 429.)

In all studies of Transpiration it is desirable to compare the rate of evaporation from the leaf with purely physical evaporation under the same circumstances. This is done with an **atmometer** or evaporation meter. Many forms of this apparatus are possible, using such evaporating surfaces as paper, porous porcelain or linen. One of the most convenient is the differential thermometer, which gives a measure of the evaporating power of the air. This is directly proportional to the " saturation deficit " of the air, *i.e.*, the amount of water vapour needed to produce saturation under the given conditions.

The differential thermometer (Leslie's) [1] consists of a U-tube of narrow bore containing a coloured liquid. Liquid Paraffin coloured with Sudan III is best. To each end of the U-tube is attached a small bulb of thin glass, either fused on or attached by pressure tubing. Care must be used to see that the liquid in the two arms remains level when the bulbs are attached. Wrap one thickness of muslin or of thin linen (old handkerchief) round one bulb and tie it firmly, with a tail hanging down which dips into a small water reservoir. Evaporation reduces the temperature in the covered bulb and causes the liquid to rise in that limb by an amount proportional to the rate of the evaporation. This gives useful comparative readings. If necessary the actual rates of water loss can be standardized by calibrating the instrument against water evaporating from an open dish which is weighed at intervals. The result can then be expressed as equivalent to grams of water per hour per square decimetre of surface.

E. COMPARISON OF STOMATAL AND CUTICULAR TRANSPIRATION

This may be done comparatively with Cobalt chloride paper, but for a quantitative result we must use the apparatus shown in the figure.

1. The two small bell jars are luted carefully with soft wax to the upper and lower surface of a large smooth leaf (Fig. 71). Resin cerate or anhydrous Lanoline (Adeps Lanae B.P.) mixed with about half its weight of liquid

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[1] May be purchased from laboratory furnishers. A modified and particularly useful type is made by Messrs Casella for meteorological purposes, see page 430.

Paraffin, makes a good seal. In each bell-glass is placed a small tube containing a drying agent, *e.g.*, Calcium chloride or Sulphuric acid, the tube and its contents being weighed. Each bell-glass is connected to a U-tube containing liquid Paraffin or Glycerine, which acts as a manometer. As water vapour is absorbed by the driers there should be a slight drop in pressure inside each bell-glass, and unless this is registered by the U-tubes, there is almost certainly a leak, which must be looked for and sealed. The water lost from the upper surface may be regarded as evaporated through the leaf cuticle, that from the lower surface as lost through the stomata. Check, by means of microscopic sections, that no stomata are present on the upper surface of the leaf used.

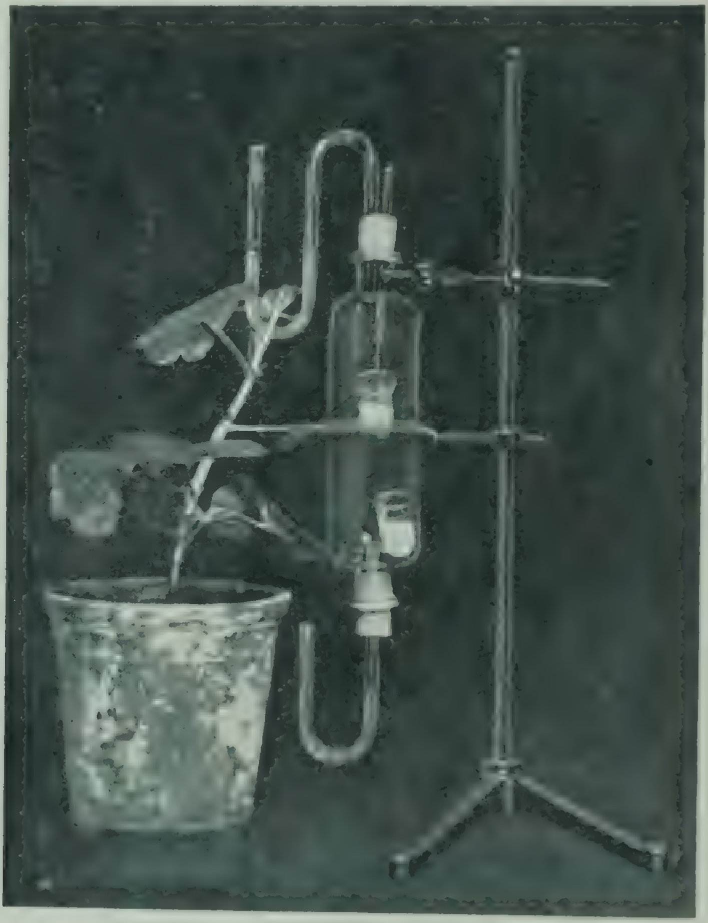

FIG. 71.—Apparatus to demonstrate cuticular transpiration.

At the end of the experiment re-weigh the tubes of drying agent. The increase in weight gives the water loss for the area of foliage enclosed by the rim of the bell-glasses. Calculate results to grams per square decimetre of surface per hour.

2. Take two fresh Apples. From one remove exactly 1 sq. cm. of skin. Weigh both to 0·1 gm. Place them on a shelf in the laboratory and re-weigh at intervals. The Apple cuticle is extremely impervious to water vapour, and the amount lost through the small skinned area is surprisingly large.

F. THE POROMETER

This instrument enables comparisons of the amount of stomatal opening to be made under varying conditions. It depends upon drawing air into and out of the spongy mesophyll through the stomata. A good type of instrument is shown in Fig. 72. It consists of a small glass cup with a broad rim, which is attached to the leaf surface in an air-tight manner by the use of a washer made of a ring of 30 per cent. Gelatine, cut with cork borers from a layer 2 to 3 mm. thick, which has been poured while warm into a Petri dish and allowed to set. In the illustration the cup is held in place by rubber bands passing over two glass hooks sealed to the sides and twisted round a microscope slide which is placed opposite the cup on the upper side of the leaf.

A short tube is fused into the bottom of the glass cup, and to this is connected a T-piece with a long vertical limb. This limb dips into a cup containing liquid Paraffin coloured with Sudan III (red dye). The third limb of the T-piece carries a rubber tube with a spring clip, and is used for sucking up the Paraffin to the top of the vertical tube.

When the liquid has thus been sucked up and the clip is closed the weight of the vertical column of liquid draws air from the spongy mesophyll of the leaf, which is replaced by air entering from the stomata outside the glass cup. The rate of fall of the liquid is therefore a measure of the rate of air passage through the stomata, *i.e.*, of their degree of opening. Note that in a dry room the stomata may close completely, so that observations should be made in a greenhouse or in a shady place out of doors.

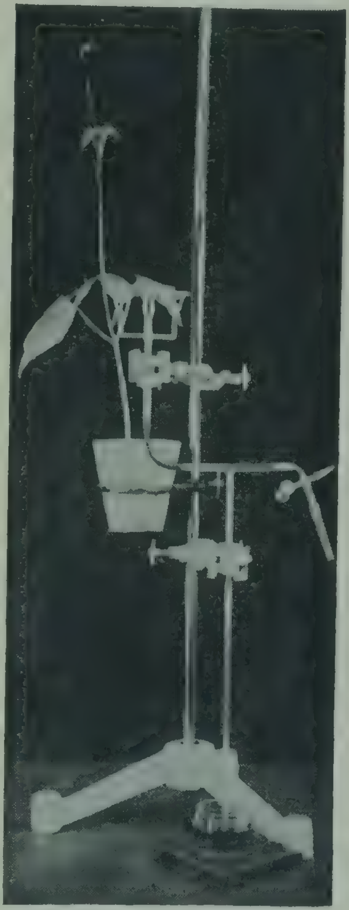

FIG. 72.—The porometer.

The experiment is best combined with the potometer measures, the porometer being attached to a leaf of the plant in the potometer. The factors which influence absorption will then be seen to have a correlated influence on stomatal opening. Test also the effect of darkness on the stomatal opening. Stomata may be fixed in their natural condition, for observation or measurement of the opening, by stripping off a small piece of the epidermis and immediately mounting it in absolute Alcohol.

To avoid, as far as possible, the error due to change of pressure during the fall of the column of liquid, the rate should always be measured over the same portion of the vertical tube. The arrangement suggested by Knight is an improvement. Here there is no vertical tube of liquid, but instead a fine bore tube is placed horizontally, connected to the leaf chamber at one end and to a large bottle of water as an aspirator at the other end. This bottle has two leading tubes through its cork, one acting as an outflow siphon, and one connected to the horizontal tube through a tap, preferably a two-way tap, to allow the admission of air. Both tubes go down nearly to the bottom of the water in the aspirator, and the rate of air entry, and hence the degree of stomatal opening, is measured by the rate of bubbling in the aspirator bottle. In this way the pressure of the air is kept constant.

G. MOVEMENT OF WATER IN A SHOOT

This can be easily followed if a solution of a non-poisonous dye is substituted for pure water.

1. Place a cut flower of white *Narcissus*, Tulip or some other large white flower, in a weak solution of Methylene Blue, *i.e.*, a deep blue but transparent solution, in a beaker. Leave for a few hours. The vascular bundles in the petals will show as clear blue lines, while the ground tissue is uncoloured. Light Green or Basic Magenta may also be used successfully.

2. Place leafy shoots of various plants, if possible with and without roots, in a similar dilute dye solution. In this case Basic Magenta (Fuchsine) is one of the best. Trypan Red is also good. These dyes do not diffuse readily, so the staining of the vascular bundles in stem and leaf is clear. Red ink is *not* suitable. Note the time taken for the vascular bundles of the leaves to become coloured, and then cut sections of the stem at various levels. Observe the staining of the xylem alone, as seen with a hand lens, and notice whether any part of the xylem tissue is unstained. Notice also that the roots exclude the dye, which is only absorbed by cut stems.

3. Test a woody shoot in which 2 in. of bark at the lower end have been peeled off cleanly, exposing the wood. Place the lower end just under the surface of the dye solution in a beaker and note that the absence of the outer tissues makes little or no difference in the rise of water to the leaves.

4. Take a shoot of an herbaceous plant with fairly large leaves, such as Sunflower. Remove all leaves but one near the top of the shoot. Vaseline the scars where leaves were removed, and the soft young parts at the apex, so as to confine water loss to the one remaining leaf. Place the shoot in Basic Magenta in an airy, bright spot for several hours. Cut sections of the stem at various levels, beginning just below the leaf. Notice that staining is confined to the bundles supplying the remaining leaf, and that in the lower part of the stem only localized portions of the xylem are stained, which are directly continuous, upwards, with the trace bundles of the leaf.

5. Stand a shoot for some hours in a dilute solution of Indian Ink, which is a colloidal suspension. Cut thin shavings from the lower end to see how far the ink has penetrated. As a colloid it cannot penetrate cell membranes, and as even vessels have occasional transverse walls, penetration is very limited.

6. Cut two woody shoots of Cherry Laurel or Privet. Place one in water and dip the end of the other into melted Vaseline or Lanoline coloured with a little Sudan III (red). After an hour in the melted material, take both branches out of their fluids and lay them on a table till the Vaseline is quite cold. Shave a fresh surface on each branch and place them both in a solution of a blue dye such as Soluble (Aniline) Blue. After an hour or two compare the progress of the dye in the shoot with blocked vessels and in the normal shoot.

7. Take a stout woody branch of Privet or a similar shrub and make a cross cut in the stem with a fret-saw, a little more than half-way through. Turn the stem round and make a similar and equal cut, on the opposite side and about 2 cm. from the first cut. The two cuts should slightly overlap one another. Support the shoot vertically in a clamp and insert a piece of tin-foil into each cut to prevent liquid passing directly across it. Now dip the lower end into weak Magenta solution and leave for some hours. Peel off the bark between the cuts with a sharp scalpel and notice how the dye has spread across the stem at this point. If a stem treated in this way is tested in a potometer it will be found that, although there is an initial drop in absorption when the cut is made, the rate recovers somewhat later, due to the increased water tension or "negative pressure" in the upper part of the shoot.

H. PERMEABILITY OF WOOD TO WATER

The presence of vessels in angiospermic wood greatly reduces the resistance to water flow. Compare samples of wood in freshly cut branches of a Dicotyledon, such as Hazel or Ash, with the wood in a branch of a Gymnosperm such as Yew. The latter, because of its smooth bark, is better than Pine or Fir, which may, however, be used if necessary.

1. Place a piece of Yew stem, about 2 in. long, in water for about six hours until it is thoroughly soaked. Hold it vertically. No water runs out at the lower end, because air cannot enter the wet tracheids at the top. Place a drop of water on the top. It is absorbed and, for every drop taken in, another drop escapes below.

2. For direct comparisons of the permeability of wood we require a manifold tube, that is to say, a straight tube with several side branches fused on (Fig. 73). This may be built up of connected T-pieces if necessary. One end of the straight tube is closed or connected to a manometer and the other to an air pump. Metre lengths of glass tubing of about 5 mm. bore are connected to the side branches, and to the lower end of each is connected, by rubber tubing, a piece of a woody shoot about 6 in. long. Dip the end of the shoot into water and start the air pump, at first very slowly. Water will rise through the wood and its rate of rise in the long tubes gives a direct measure of permeability. If we know the working pressure and the area of the wood in each shoot we can express the permeability numerically in cubic centimetres of water per square centimetre per atmosphere, assuming

an equal length of wood is used in each case. If only two specimens are compared they may be attached to the arms of a Y-tube instead of using the manifold as described.

Compare a deciduous Angiosperm like Hazel with an evergreen Angiosperm such as Holly, and contrast these with a Gymnosperm such as

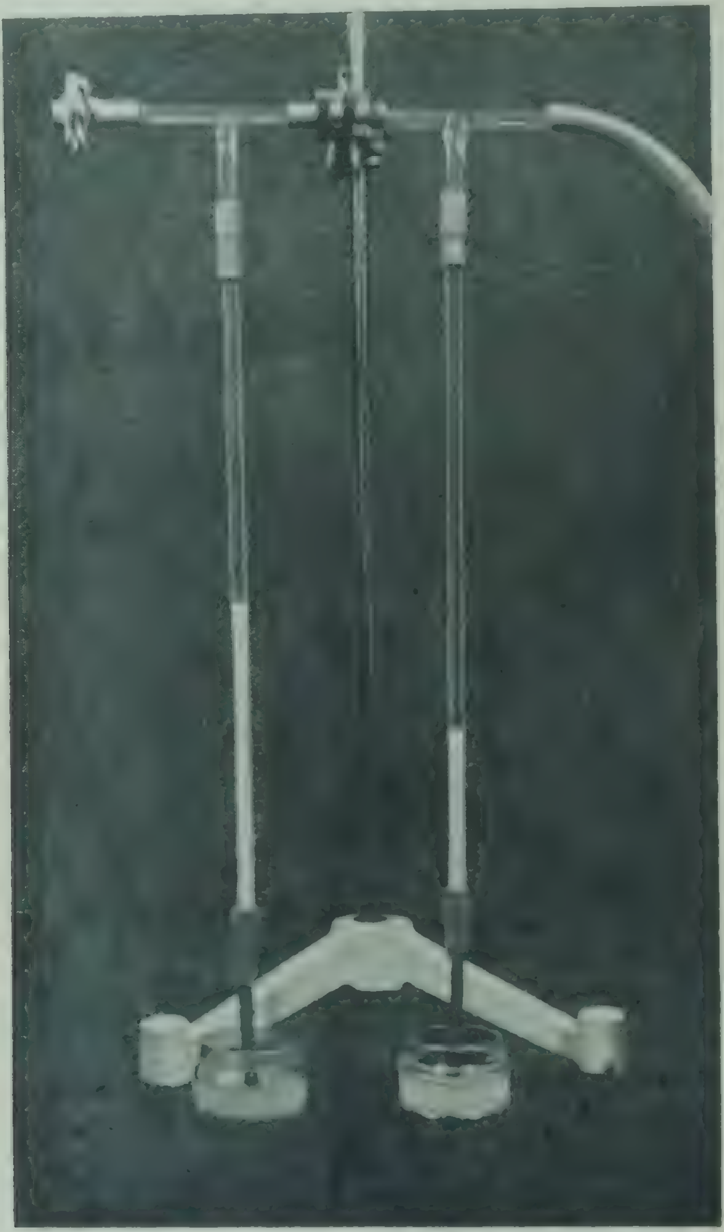

FIG. 73.—Apparatus to compare the relative permeability of woods.

Yew, first in its natural state and then with the protoxylem vessels blocked. The latter is accomplished by gouging out a small cavity in the centre of the stem at each end and filling it with hot wax.

As precautions against leakage of air it is well to wire each specimen carefully to the tube and to rub the bark over with Vaseline to close up the lenticels.

3. Remove the water from beneath each specimen and turn on the pump. Does air penetrate easily through the wet wood? If not, or if it penetrates in some cases only, suggest an explanation.

4. With a piece of angiospermic and of gymnospermic wood together in the apparatus, test the effect of slowly cutting across each with a fret-saw. Note how much you have to cut through in each case before a diminution in the rate of flow becomes evident. A considerable portion of the xylem in all cases is non-functional as regards water flow.

5. A very satisfactory way to demonstrate the difference between angiospermic and gymnospermic wood is by the Gas Test. Take a short length of woody stem and attach tightly a piece of rubber tubing. Fix the other end of the tubing to the gas tap and turn on the gas. After a short while, attempt to light the gas at the end of the piece of wood. In the case of angiospermic wood it will be found possible to do this, because the vessels are continuous throughout the length of the piece of stem. In the case of gymnospermic wood the same is not true, for the relatively short tracheids prevent the passage of coal gas through the wood.

J. ROOT PRESSURE

Get a small but healthy pot plant of *Fuchsia*, Balsam or *Pelargonium*. If you have a garden to work in, choose a vigorous growing shoot of some herbaceous plant with a fairly stout stem. Evening Primrose, Great Willow Herb or Nettle all do well. In any case, cut it off with a razor about 2 in. from the ground. Slip on to the stump a piece of pressure tubing which makes a close fit, leaving about 1 in. of tubing free (Fig. 74). Fill this with water. At first this will probably be absorbed. Repeat the filling with water till absorption ceases. Now push into the rubber tube a piece of straight glass tubing about 1 metre long and of narrow (1 mm.) bore. The water will be forced a short distance up the tube. Mark its level. Give the plant a good watering and observe the rise of water in the tube from time to time. The rise should continue for some days. What brings it to an end? Are the roots dead? Leave the plant for a while and you may see it start to grow again.

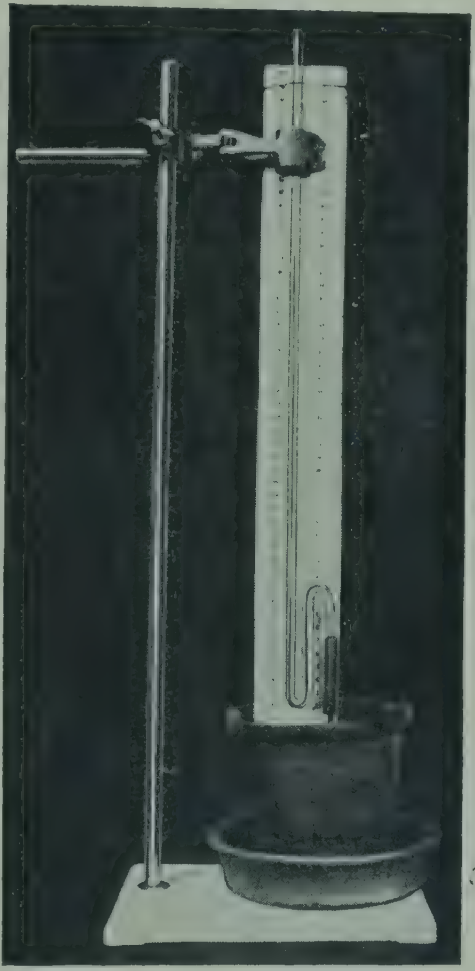

FIG. 74.—Apparatus to measure root pressure.

Root pressure is probably of minor significance in supplying the shoot with water. Its occurrence is uncertain and usually seasonal. The main motive force in absorption is no doubt the suction force due to the tension of water in the xylem elements.

K. ASKENASY'S EXPERIMENT

The loss of water from an evaporating surface may cause the development of a considerable tension in the water beneath the surface, and this is a powerful agent in drawing water up the stem towards the leaves.

1. Take a thistle funnel with a long stem and clean it carefully. Mix some Plaster of Paris (Calcium sulphate) on a glass plate to a stiff paste with water, taking care to work the plaster and water thoroughly together. Mould it into a cake, about 1 cm. in thickness, and press the thistle funnel mouth firmly into it so as to cut out a disc. Cut away the rest of the cake and pour a few drops of water on to the plate so as to allow the funnel, with the enclosed disc of plaster, to be slid smoothly off the plate. Set aside for a day to harden then carefully paint round the edge of the disc with shellac or other sealing agent to prevent leakage of air.

Carefully fill the funnel, through the stem, with freshly boiled and cooled water. This may best be done by inserting a thin wire into the stem and pouring the water along the wire from a pipette. When quite full place a finger over the end and invert it into a small trough of Mercury. Clamp the funnel vertically. As water evaporates from the surface of the plaster disc, Mercury will rise in the stem of the funnel, and the cohesion of the water to the plaster is sufficiently strong to support a considerable column of Mercury. If an extension tube is fitted to the funnel stem it can be shown that the Mercury will rise to well over barometric height (760 mm.), thus showing that it is not supported entirely by atmospheric pressure, but by the tension of the water above it.

2. An alternative arrangement, which is sometimes easier to secure, is to attach a porous pot, which has been well boiled to saturate its pores with water, to the top end of a long glass tube which has been bent over so that the pot hangs downwards. A good rubber cork must be used for the connection. The end of the glass tube is dipped into boiled water in a trough and filled by siphoning. The pot is then plunged under the water and the connection is made while it is submerged, so as to exclude all air bubbles. This is essential for success. The lower end of the glass tube is inserted in a dish or bottle of Mercury as before.

3. The same effect, due to transpiration, may be shown by attaching a freshly cut leafy shoot with rubber tubing to a long glass tube which is filled with water and inverted into Mercury as before.

4. Take a large seedling, such as a Broad Bean, which has been dug up with its roots and left lying till the leaves are wilted but not withered. Plunge the lower end under water coloured with an aniline dye and cut through the base of the stem under the liquid. If the stem is then split open

lengthways the colour will be found to have run up the bundles of the cut stem for some distance, almost instantaneously, thus demonstrating the existence of a strong tension or "negative pressure" in the water channels.

Section 4. Assimilation

A. FORMATION OF STARCH IN THE LEAF

Starch is the visible product of photosynthesis in the great majority of green plants, and its presence may be regarded as the primary evidence of the process.

1. To demonstrate the presence of Starch in a leaf we must first remove the Chlorophyll, the colour of which masks the starch reaction. Plunge the leaf for a few moments into actively boiling water. This kills the cells and leaves the protoplasm highly permeable. Transfer the leaf to warm Alcohol which will dissolve the Chlorophyll and leave the tissues white. Some species of plants never blanch completely by this method and a trial should be made of several sorts. Now place the blanched leaf in a solution of Iodine in aqueous Potassium iodide solution. This is best used in a white photographic dish or a white plate which shows up the dark bluish-black colour produced by the reaction of starch and Iodine.

Microscopically, the presence of Starch grains and their distribution in a leaf are best seen by mounting portions of thin leaves in a solution of Iodine in Chloral hydrate (Chloral hydrate 8 parts, water 5 parts, Iodine enough to give a good brown colour) and leaving for twelve to twenty-four hours. This mixture dissolves the protoplasmic structures, thus clearing the tissues. Starch grains swell and turn blue-black. Leaves of species of *Impatiens* or those of thin-leaved water plants are particularly suitable. A similar result may be obtained by soaking in Eau de Javelle, followed by staining with Iodine solution.

2. Test variegated leaves as above. The presence of Starch will be found to coincide with the presence of Chlorophyll.

3. Cut a simple stencil pattern out of black paper and place it on a leaf. *Pelargonium* or Tobacco plants answer very well. The black paper should be large enough to leave a margin standing out on both sides of the leaf. Place an equal-sized piece of black paper below the leaf and clip the two together at the corners with paper clips. The leaf is thus screened from light except for the portion exposed through the openings in the stencil. Expose to light for several days and then test with Iodine as described above. The shaded part between the papers had access to Carbon dioxide from the air, as the paper covering is not air-tight, but it has nevertheless lost its Starch, which is only found where light has penetrated the stencil openings (Fig. 75). Some leaves, *e.g.*, Garden "Nasturtium" (*Tropaeolum*), form Starch so richly that prints in Starch from photographic negatives may be obtained in this way. The Starch-Iodine colour soon fades, but the

preparations may be kept in Alcohol and repeatedly "developed" by the application of fresh Iodine solution.

4. Colour Screens may be applied to leaves to test which parts of the spectrum are effective in photosynthesis. This is best done with coloured Gelatine light filters. Fig. 76 shows the light transmission of some suitable filters of the Wratten series (Kodak Ltd.), which may be bought very cheaply in the form of unmounted Gelatine. They should be cut into pieces about ½ in. square and arranged in a row on a glass plate, with a black card mask cut to fit over them, covered with a second glass plate and bound with adhesive tape at the edges. One space, of similar size, should be left without a colour screen as a white-light control.

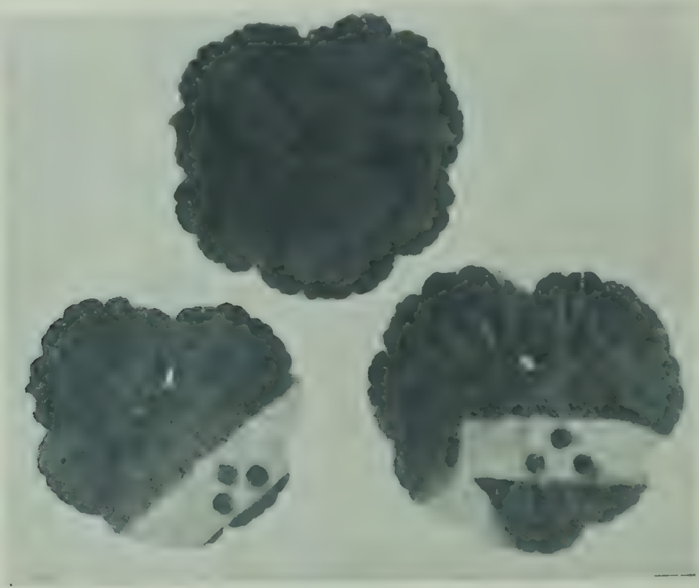

Fig. 75.—Zonal Pelargonium (Geranium) leaves after screening.

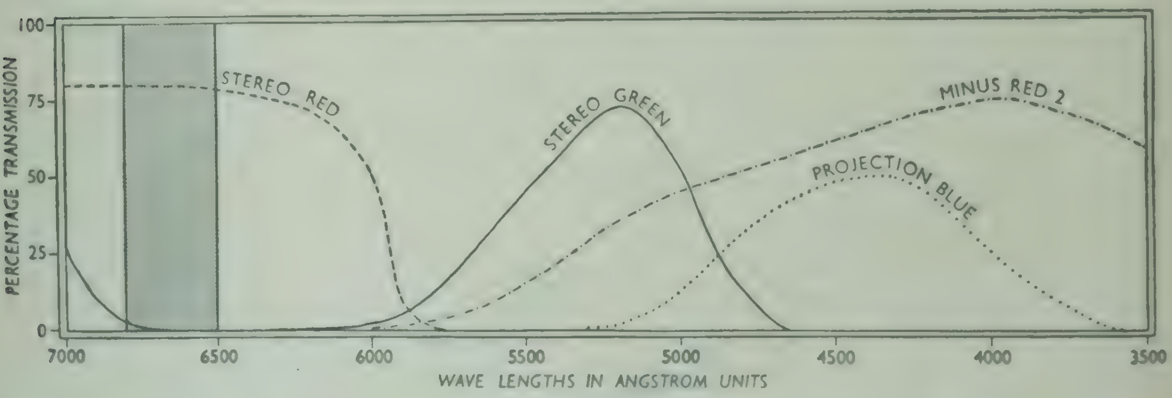

Fig. 76.—Graphs of the relative absorptions of coloured screens.

The four screens selected have the following characteristics :—

No. 26, Stereo Red.—This gives a high transmission over the red-orange portion of the spectrum in which the main Chlorophyll absorption lies and cuts sharply at the yellow, wave-lengths below Å 5850 being completely cut out. The total transmission is 22 per cent. of white light, the maximum (79·4 per cent.) being between Å 7000 and Å 6500. It produces almost as much Starch as white light.

No. 55, *Stereo Green.*—This is a screen which has a transmission closely resembling that of Chlorophyll itself and cutting out practically all the light which Chlorophyll absorbs. The result is that no Starch formation takes place below this screen. The total transmission is 31 per cent., the maximum being 72·5 per cent. at Å 5200.

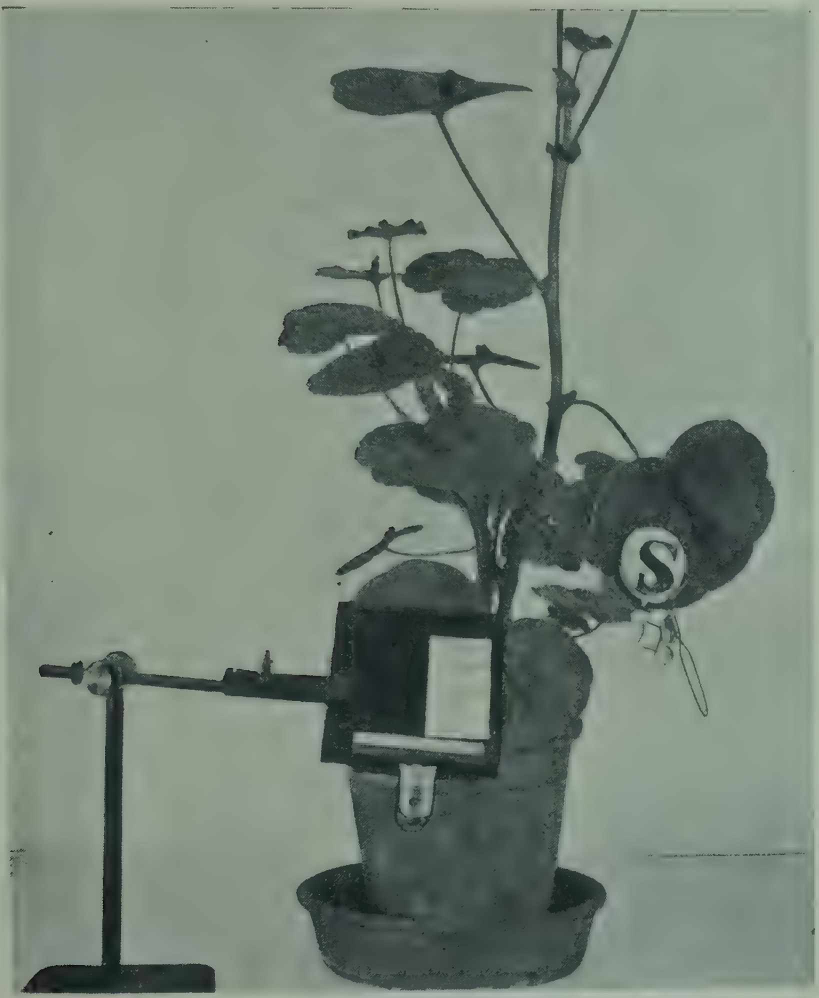

FIG. 77.—A plant with coloured screens attached.

No. 47, *Projection Blue.*—This is the most transparent of the available pure blues, though as blue and violet light have very low luminosities, its total transmission is only 2·9 per cent. It has, however, a maximum transmission of 49 per cent. between Å 4400 and Å 4300. Slight Starch formation takes place below this screen.

No. 43, *Minus Red* 2.—This is included because of its specially high transmission in the blue-violet region (74 per cent. at Å 4000), but it also passes a small amount of green and a very small amount of yellow. These rays are not, however, absorbed by Chlorophyll, so that the slight starch formation below this screen may be traced to the absorption of the violet rays by chlorophyll. The colour of this screen is sensitive to heat. Its total transmission is 12·5 per cent.

Expose a leaf under the screens for several days, the lower surface being protected by loose black paper backing, and then develop with Iodine as above (Fig. 77).

B. ASSIMILATION OF CARBON DIOXIDE FROM THE AIR

1. Take two small potted plants of healthy growth, *e.g.*, Wallflowers, and test one leaf of each of them for Starch (see p. 327). Use only plants which show satisfactory amounts of Starch present.

Fit up two tubulated bell jars large enough to cover the plants used. The tubular opening at the top should be fitted in each case with a rubber stopper or a waxed cork through which passes the narrow end of a Calcium chloride tube, or else a glass tube connected to a U-tube. One should be filled with granulated Soda lime to absorb Carbon dioxide and the other with small pieces of pumice, gravel or glass wool, which will restrict the entry of air to about the same amount as that passed by the Soda lime.

Place the bell jars on two plates of glass. Place the plants under them, and under the one fitted with the Soda lime tube place, additionally, a small basin of strong Potassium hydroxide solution, while a similar dish containing water is placed under the other. Brush melted Vaseline round the rim of each jar to seal it to the glass plate. The two plants are now under identical conditions, except that in one case the air under the bell jar is deprived of all its Carbon dioxide.

Leave the jars standing for several days in a bright, but not too sunny, spot. Take leaves from each plant and again test for Starch. The plant deprived of Carbon dioxide is unable to manufacture any, and if left long enough under such conditions will die of starvation.

2. *Moll's Experiment.*—A very simple way of making a similar demonstration is the following. Take a glass jar and fit it with a cork stopper. Cut this in half lengthwise and place a long leaf, such as Cherry Laurel, between the halves, so that they grip it about midway along. Place the jar horizontally and pour a little Potassium hydroxide solution into it. Fit the cork in place so that half the leaf is inside the jar and half (the stalk half) outside. Place a little water for the stalk to dip into and leave the experiment for some days. The half leaf inside the jar will be found to be devoid of Starch.

C. EVOLUTION OF OXYGEN IN PHOTOSYNTHESIS

1. *The Elodea Experiment.*—Oxygen is visibly given off in bubbles from submerged water plants like *Elodea.* The experiment, though simple, needs careful control.

Select a few healthy young shoots of *Elodea.* Tie the stems into a bundle with thread, being careful not to crush them. Plunge them into a large beaker of water at 25° C. and cover closely with a glass funnel, upside down, which fits inside the beaker (Fig. 78). Over the stem of the funnel invert a test-tube full of water. In bright light, and especially in the spring and summer months, bubbles will be rapidly given off and will collect in the test-tube. This gas is in part Oxygen set free by photosynthesis, but it is never pure. On a dull day it may be only about 30 per cent. Oxygen, and under the best conditions it scarcely rises to 50 per cent.

To get the best results a ready supply of Carbon dioxide and Oxygen must be available in the water, otherwise the slow diffusion of gases from the water surface will limit the action. Saturate the water at 25° C. with air by prolonged and vigorous shaking or by bubbling air through it for some time and dissolve in it 1 per cent. of Potassium (not Sodium) bicarbonate. The latter will provide Carbon dioxide by dissociation.

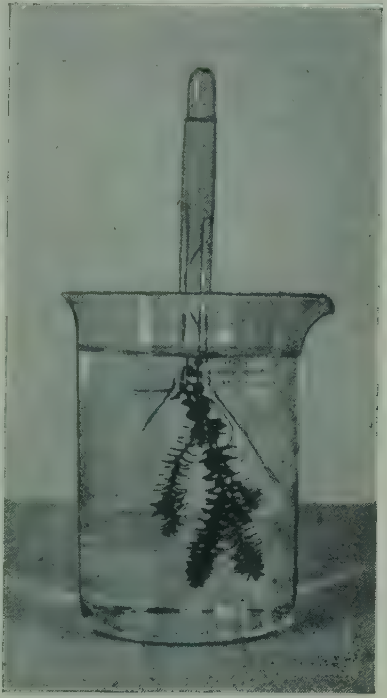

Fig. 78.—Apparatus to demonstrate the evolution of Oxygen by *Elodea*.

If the percentage of Oxygen in the gas collected is up to 50 per cent. it will relight a glowing splinter, but it sometimes fails to do this. To analyse the amount of oxygen present, use a glass tube bent into a U-shape, with one limb turned outwards and fitted with a rubber teat. Use this as a sampler tube to withdraw a bubble of gas from the test-tube, sucking in first a drop of water and then a bubble of gas, then a second drop of water. Withdraw the sampler and measure the length of the gas bubble. Turn the sampler upside down and dip the free end into a mixture of 25 per cent. Potassium hydroxide with 7 per cent. Pyrogallol freshly made. Carefully expel the second drop of water and draw in the Pyrogallol solution in its place. Re-measure the length of the bubble. The ratio of the two lengths will give you the percentage of Oxygen present in the gas.

The same set-up may be used for testing the effect of external factors on photosysthesis, by timing the rate of the evolution of bubbles. The reciprocals of the times for five bubbles under two different conditions give a direct measure of the relative rates of photosynthesis in the two cases.

(*a*) Try different light intensities at different distances from a 50 watt electric light.

(*b*) Vary the Carbon dioxide concentration available by varying the bicarbonate strength. Potassium bicarbonate yields 22 per cent. of its weight as CO_2 on dissociation.

(*c*) Test coloured lights by screening with sheets of coloured glass or Gelatine in a strong light.

(*d*) Test the temperature effect by stirring warmed bicarbonate solution into the beaker and reading the increased temperature with a thermometer.

(*e*) Increase the Carbon dioxide concentration progressively, under two different light intensities, and plot the reciprocals of the times for five bubbles against the Carbon dioxide or the bicarbonate concentrations in each case. These curves show at what concentration of Carbon dioxide the light becomes the limiting factor (Prime's method).

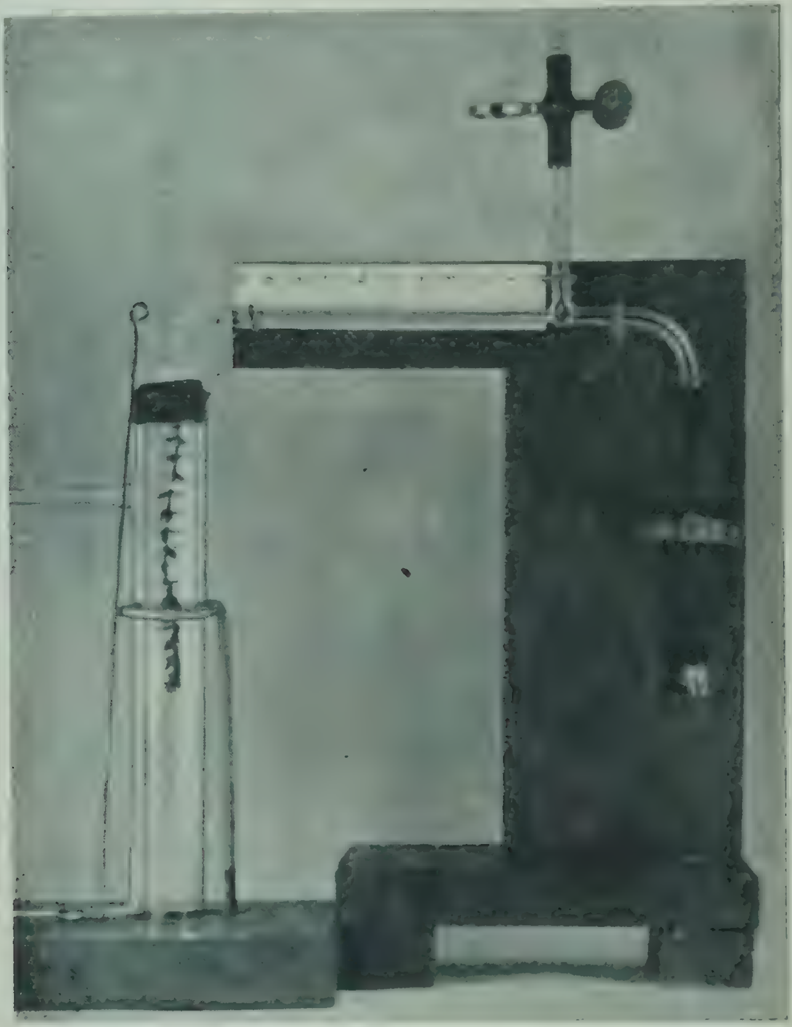

Fig. 79.—Apparatus to measure the Oxygen evolved by *Elodea.*

2. *Quantitative Estimation of the Oxygen Evolved in Photosynthesis of Elodea.*—For this purpose the apparatus described by Dr L. J. Audus may be used. It consists (Fig. 79) of a glass tube about 1·5 cm. diameter which is pierced by a number of large holes. The upper end is sealed into a capillary tube of 1 mm. bore which is bent at right angles and forms a microburette to which a graduated scale is attached. To the end of this tube is sealed a T-piece. In the upper tube is blown a small glass bulb to act as a gas trap, and the end of the tube is closed by a pinch-cock attached to a piece of rubber tubing. The lower arm of the tube is also fitted with a

piece of rubber tubing which is closed by a short length of glass rod. A second pinch-cock is attached to this piece of rubber tubing.

The shoot of *Elodea* is introduced into the perforated tube with the aid of a piece of cotton passing through one of the top holes, taking care that the cut end is uppermost. The whole tube is then lowered into an outer jacket containing the experimental solution, while the temperature of this solution can be controlled by enclosing it in a water jacket through which water as a constant temperature is circulating. The whole apparatus is filled with the experimental solution by applying suction to the upper rubber tube in the T-piece.

The apparatus is placed in a bright light and the bubbles of evolved gas collect in a large bubble at the junction of the perforated tube and the capillary tube. After a convenient interval of time this bubble is drawn into the microburette by operating the lower pinch-cock. The length of the bubble is measured against the scale, and since the diameter of the tube is known, the volume of gas evolved may be calculated.

After measuring the bubble, further manipulation of the lower pinch-cock draws the bubble to the end of the capillary tube, when it escapes into the gas trap, while at the same time another bubble is drawn into the microburette.

Try the effect of altering the same factors influencing photosynthesis as have already been suggested in Experiment 1.

3. *Kolkwitz' Method.*—The simplest of the various chemical methods of demonstrating the evolution of Oxygen is that with reduced Indigo Carmine (Sodium indigo sulphonate).

Indigo Carmine is dissolved in tap water,[1] about 0·1 gm. per litre. This gives a bright blue solution. Make up an approximately 10 per cent. solution of Sodium hydrosulphite ($Na_2S_2O_4$) in the same water. Add the latter, very carefully drop by drop, to the former until it is completely reduced to Indigo White. It is very important to avoid an excess of even a single drop of the hydrosulphite. The reduction should be carried out in a large beaker, using several hundred cubic centimetres of the Indigo solution, and after reduction the solution must not be shaken or disturbed in any way.

Gently suck up about 20 c.c. of reduced Indigo in a pipette and fill a test-tube with it, putting the pipette down to the bottom of the tube and avoiding contact of the solution with air as much as possible. If any considerable blueing of the fluid takes place during this process add another very small drop of the hydrosulphite. Now insert a small piece of young *Elodea* shoot into the test-tube, make sure that the tube is perfectly full of liquid and then invert it into the beaker (Fig. 80). Place in a bright light (Fig. 81), and even in a few minutes clouds of blue will be seen coming from the *Elodea* leaves, due to the re-oxidation of the Indigo by the Oxygen set free in photosynthesis. A control placed in the dark should show no

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[1] The introduction of a few cubic centimetres of Soda water improves the experiment by supplying Carbon dioxide.

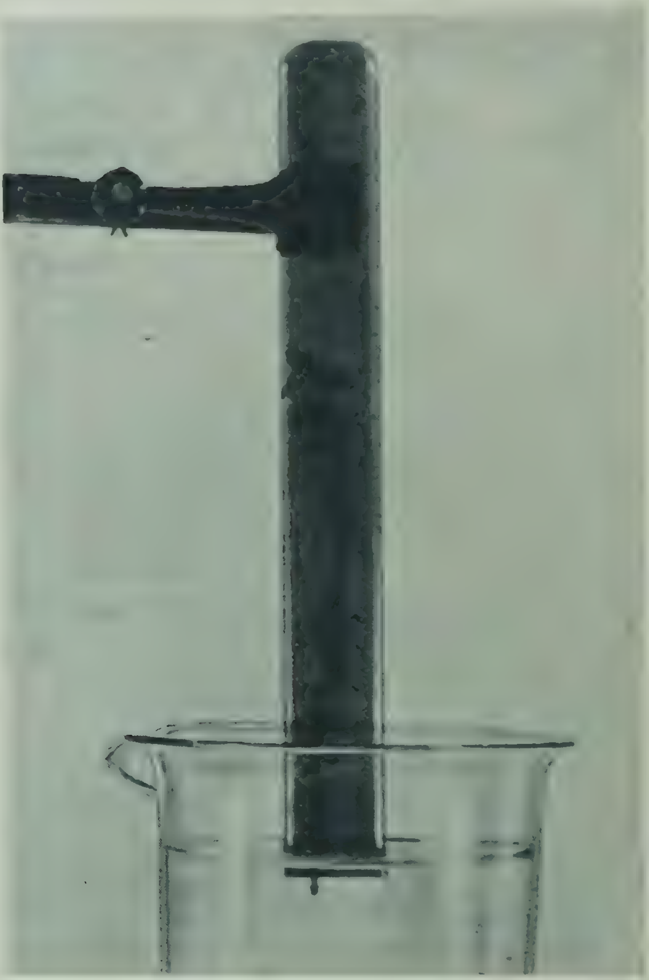

FIG. 80.—The Indigo-carmine method of demonstrating the evolution of Oxygen by the leaves of water plants.

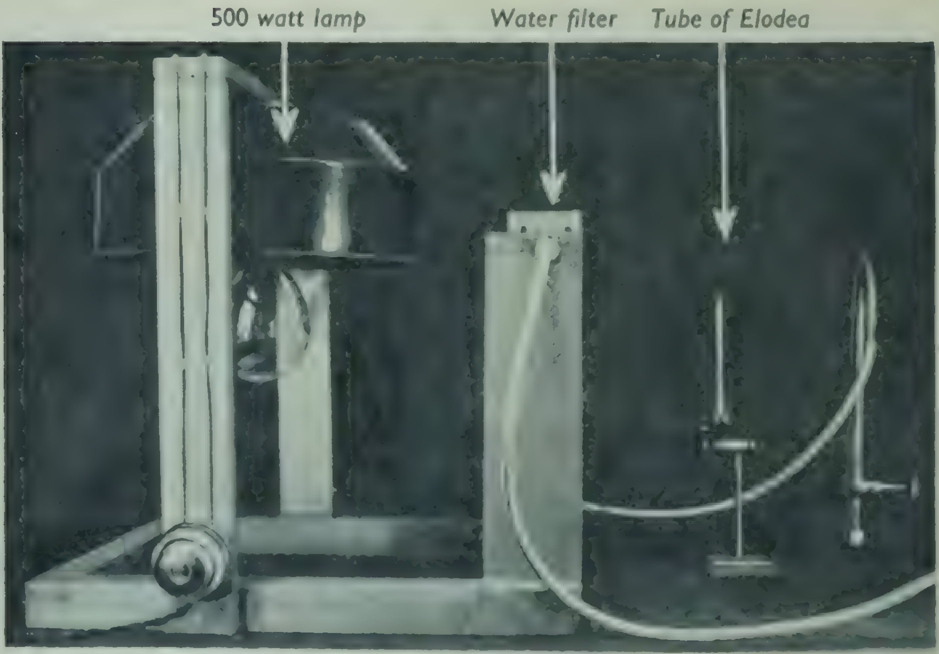
500 watt lamp
Water filter
Tube of Elodea

FIG. 81.—Apparatus to illuminate plants during photosynthesis experiments

oxidation, nor should roots or other non-chlorophyllous parts, even when exposed to light.

The duration of the experiment may be prolonged by using dilute (5 to 6 per cent.) Gelatine solution to dissolve the Indigo Carmine.

D. GAS EXCHANGE IN PHOTOSYNTHESIS

1. *Priestley's Experiment.*—Get three wide-mouthed bottles, three shallow dishes or saucers and a large basin of water. Cut a piece of cork, smaller than the mouths of the bottles, and stick a small piece of candle on it so that it will float upright in the water basin. Also attach a piece of thread to the cork. Light the candle floating on the cork and invert one of the bottles over it, with the mouth below the surface of the water. When the candle has produced about 3 per cent. of Carbon dioxide in the air in the bottle it will go out. Pull it away with the thread and push a leafy shoot up into the bottle. Slip a saucer under the mouth of the bottle and lift it out of the basin, keeping the mouth under the water in the saucer. Stand it in a bright light. Treat the other two bottles similarly, but in one case place the bottle and leaves in the dark, and in the third do not insert any leaves. This latter is the control.

After a few days of exposure to light return the bottles to the water basin, withdraw the plants and slip a plate of glass over the mouth of each bottle. Place them mouth upwards on the table and test the enclosed gas by lowering into each bottle a piece of lighted candle. Only in that which contained leaves and stood in the light has the Carbon dioxide originally formed by combustion been removed, so that the candle will continue to burn.

2. *The Ganong Photosynthometer* (Fig. 82).—This apparatus is delicate and somewhat complex, but it gives very accurate results and may be recommended where good careful work is possible. The following description is shortened from the original. To produce an easily measurable result it is necessary to increase greatly the natural amount of Carbon dioxide in the air, up to 5 or even 10 per cent. Push a young leafy shoot into water in a graduated cylinder and cut off exactly 2 c.c. of material. Garden Nasturtium is one of the best plants for the purpose, and Wallflower is good in the spring. Place this in the glass bulb of the apparatus. Invert the measuring tube of the apparatus, close the stopcock, and fill with water up to a mark corresponding to the desired percentage of Carbon dioxide. The apparatus when complete holds exactly 100 c.c. of air and 2 c.c. of plant. Place the hollow stopper on the tube, close the tap, fill the hollow with water, closing it with the hand, invert into a basin of water, clamping it so that the water is level with the bottom of the stopcock bore. Open the lower tap and connect the top of the tube with a Carbon dioxide generator which is open and delivering gas freely. Open the top tap cautiously to admit Carbon dioxide and close it again when the water has been driven down to the level of the water outside. Close both taps and transfer the whole tube to the top of the glass globe. Allow extra air pressure to escape through the holes

in the side of the stopper, then give it a twist to seal it off. Open the lower stopcock and the enriched gas in the tube will diffuse into the bulb. Place the apparatus in a bright diffused light for several hours, then shut the lower stopcock, remove all the top parts to a basin of water and remove the hollow

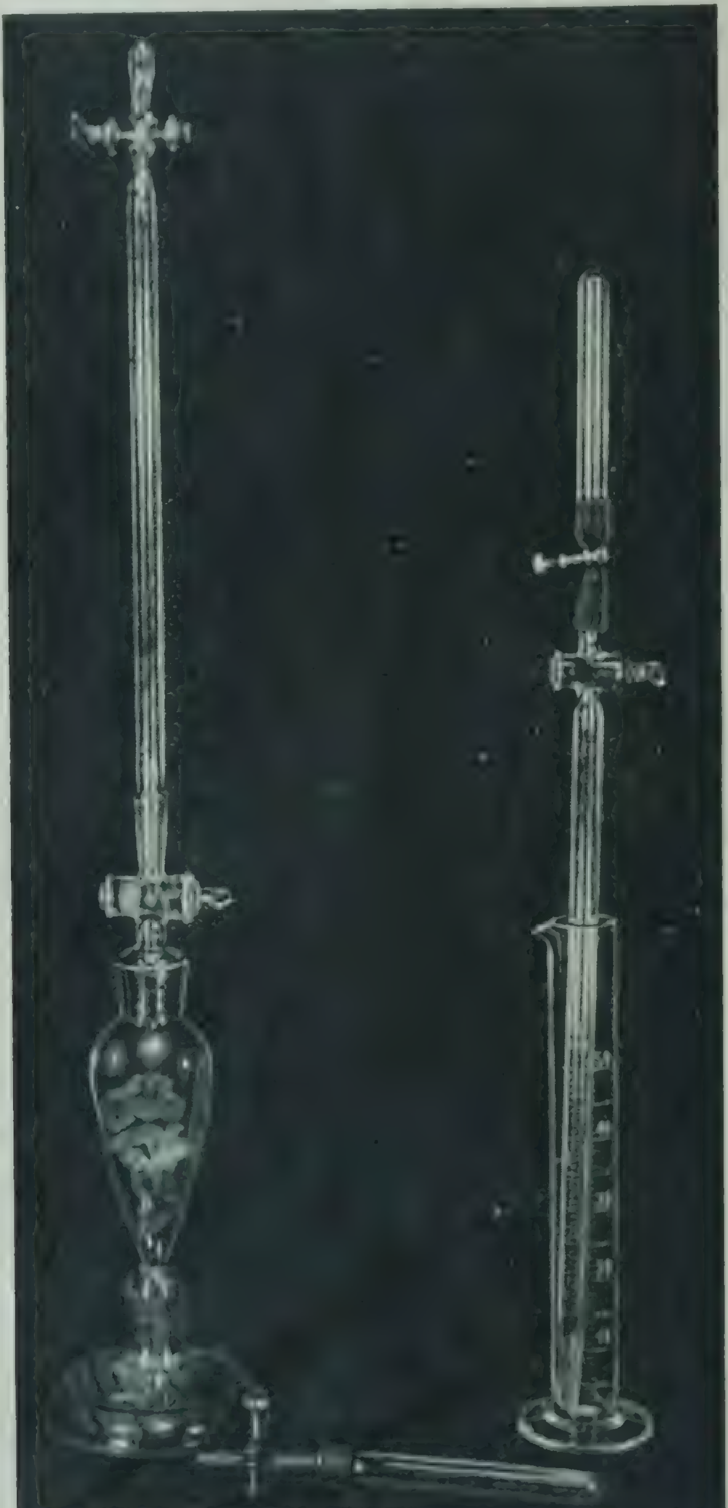

Fig. 82.—The Ganong photosynthometer.

stopper under water. Fix the graduated tube with the zero mark exactly at the surface of the water and open the top tap gently to let the water rise inside the tube exactly to zero.

Fill one of the test-tubes completely with 30 per cent. Potassium hydroxide. Slip the rubber connection on to the bottom of the graduated tube, lift all out of the water, open the spring clamp and allow the Potash to flow into the tube. Shake thoroughly, drain back into the test-tube and shut the clamp. Place the end under water with the zero at surface level again and slip off the test-tube. The water will now rush up to a mark which will show how many cubic centimetres have been absorbed by the Potash, and hence what percentage of Carbon dioxide was present. Repeat the test with alkaline Pyrogallol (Pyrogallol 1 part, Potassium hydroxide 5 parts, water 30 parts) to absorb Oxygen. The diminution in the amount of Carbon dioxide and the gain in Oxygen should tally closely. Some slight solution of Carbon dioxide in the water used will introduce a slight error.

E. DRY WEIGHT METHOD OF MEASURING PHOTOSYNTHESIS

The formation of Starch represents the addition of solid weighable material to the leaf, the amount of which can be measured.

1. *Sach's Half-Leaf Method.*—In the early morning take a healthy plant before assimilation has started and, without cutting across the petiole, split several leaves down the middle with a razor, so that the two halves shall be as nearly equal in size as possible. Remove one half from each leaf and dry in an oven at 110° C. They should be tested at least once for constancy of weight by placing them in a desiccator to cool and weighing. Return them to the oven for another half-hour and weigh again. No further loss should be measurable if they are completely dry. In the evening cut off the other halves of the leaves, dry and weigh them. The increase represents the addition of Starch. Averaging a number of leaves will reduce the inevitable error, but the method is only approximate.

2. *Ganong's Disc Method.*—Using a Ganong leaf cutter or a sharp cork borer cut out a large number of discs of leaf tissue in the morning, avoiding the large veins of the leaf. Dry and weigh as above. Repeat the process in the evening. Measure the area of the discs cut and express the gain in weight in milligrams per square decimetre of foliage per hour.

F. EXTRACTION OF CHLOROPHYLL. ABSORPTION SPECTRUM

It is advisable to use, for extraction, leaves with a high Chlorophyll content. Elder leaves, grass (lawn mowings) and Ivy are all good, but the best is probably Nettle, as it can be dried without decomposition of the Chlorophyll and thus gives a stronger solution and may be stored for use in winter.[1]

About 2 gm. of chopped up leaves are placed on a filter paper and the whole inserted in a Buchner funnel, after which the material is gently sucked against the paper. About 3 c.c. of an 85 per cent. solution of Acetone is added and allowed to soak for a few minutes before being sucked through by the pump. The operation is repeated till about 20 c.c. have been sucked through. This Acetone solution is then poured into a separating funnel, and double the quantity of Petroleum Ether is added. About 50 c.c. of distilled water is gently poured down the side of the separating funnel, to avoid the production of an emulsion. In a few minutes the Ether layer separates out on top, and it is this which contains the pigments. The lower watery layers are run off and more distilled water is added in the same way and again run off in order to wash the extract. The resulting etherial solution contains all four pigments.

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[1] Fresh Nettle leaves cannot be successfully used.

The solution viewed in a thin layer is pure green, but in a thick layer it looks almost black, and examined by reflected light, especially if the light is condensed with a lens upon the surface, it shows a strong red fluorescence, so that the whole solution appears blood-red, the complementary colour to the green. This red colour corresponds to that which is absorbed by Chlorophyll, as shown by the transmitted spectrum.

This latter may be individually observed with the aid of a small direct-vision spectroscope, or a demonstration may be arranged by placing in front of the lens of a projection lantern, an opaque card with a very narrow slit cut in it and fixing a prism in the path of the ray from the slit. Turn the prism so that the projected spectrum falls upon a white paper and focus it, if necessary, by means of a convex lens interposed between prism and paper. Fill a flat glass vessel, or failing that a test-tube, with a strong chlorophyll solution and place it in front of the slit. Only deep red light (about Å 6800) is transmitted. Now substitute a diluted solution or a thinner layer of solution. A strong black absorption band in the red (about Å 6600) is easily visible and another weaker band in the orange, about Å 6200. If you have only a single prism these two bands will probably not be separated, but will appear as one broad band. They represent the principal absorption of Chlorophyll, *i.e.*, the region of the spectrum from which most light energy is derived. A good spectroscope will also show weak bands in the green region, while the blue and violet are largely, though not completely, absorbed. As these regions are not very luminous in the solar spectrum they do not, however, contribute so much energy as the orange-red absorption. Part of the orange, the yellow and the green, however, suffer little or no absorption and so contribute nothing to the energy supply for photosynthesis (see under Starch Prints, p. 327).

Add a few drops of dilute Hydrochloric acid to a Chlorophyll solution. It turns brown owing to the formation of Phaeophytin. In the spectrum there will now be seen pronounced bands in the green, an indication of the decomposition of the Chlorophyll. Neutralize the solution with chalk, then add a few drops of a copper sulphate solution. The bright green colour of Copper chlorophyll appears.

Haemoglobin absorbs chiefly in the yellow, as may be seen by examining the light transmitted between two fingers held together in front of a strong light.

G. SEPARATION OF THE LEAF PIGMENTS

The solution dealt with in the preceding section is a " raw " solution containing both Chlorophylls A and B, and also Carotin and Xanthophyll. The separation of the two green and the two yellow pigments is relatively easy.

1. Place some strong freshly prepared Chlorophyll solution in a shallow dish and hang a strip of filter paper so that the lower edge just dips into the fluid. In a few minutes the solution will have mounted into the paper, and it will be seen that a yellow zone goes in advance of the green, the former being chiefly Carotin, which is not readily adsorbed. It may also be possible to distinguish that the upper edge of the green zone is bluish in tint (Chlorophyll A) and the lower green portion yellowish (Chlorophyll B).

2. To separate the Chlorophyll pigments from Xanthophyll and Carotin shake up 5 c.c. of the etherial extract with 2 c.c. of a 30 per cent. solution of Potassium hydroxide in pure Methyl alcohol. After the green colour has reappeared add 10 c.c. of water and a little more Petroleum Ether. Shake the tube and allow to settle. Two layers separate, the upper layer containing the Xanthophyll and Carotin, while the lower layer contains a mixture of saponified Chlorophylls.

A simpler and more rapid separation of the pigments may be obtained by using fresh grass which has been steeped for a few moments in boiling water and then squeezed dry. The pigments are extracted with warm 95 per cent. Alcohol. This raw extract is transferred to a test-tube to which an equal quantity of Benzene is added. The tube is shaken strongly and then allowed to stand, when the Benzene layer separates out above as a dark-green layer containing the two Chlorophylls, while the lower layer of Alcohol retains the yellow Xanthophyll and Carotin.

3. *Tswett's Chromatogram.*—The only relatively simple way of separating all four pigments is by making use of the differences in their adsorption upon a solid substrate, as in No. 1, above. This can be accomplished by the filtration of a " raw " leaf extract through a column of precipitated chalk. Put a small wad of glass wool in the bottom of a Calcium chloride tube and over it carefully pack about 2 in. of precipitated chalk, adding it little by little and pressing it down with a plunger so as to get a perfectly even medium for filtration. The chalk should be thoroughly dried in an oven for two hours beforehand. The success of the experiment depends largely on the dryness and homogeneous packing of the chalk. Leaf tissue (preferably dry) is extracted in the cold with a mixture of Alcohol 1 part, Petrol Ether 10 parts. Some powdered chalk may be added to the leaf tissue and the whole ground together in a mortar. Pour off the solution into a stoppered separating funnel and add gradually twice its volume of water, shaking gently and carefully for a minute or two. Allow the mixture to settle. All the Alcohol will separate with the water layer, and may be run off through the tap. Run off the Petrol Ether extract into a stoppered bottle with a layer of Calcium chloride on the bottom and let it stand so for a day to dry.

Mount the packed tube of chalk in the cork of a filter flask and attach the side arm to an air pump (Fig. 83). Pour on gently from a pipette a little pure Petrol Ether and suck it through slowly, adding more if needed to ensure that the chalk layer is thoroughly saturated with it. Then add the Chlorophyll extract in the same way and let it filter slowly. The constituents will form superimposed coloured layers as follows (Fig. 84), reading from the top downwards :—

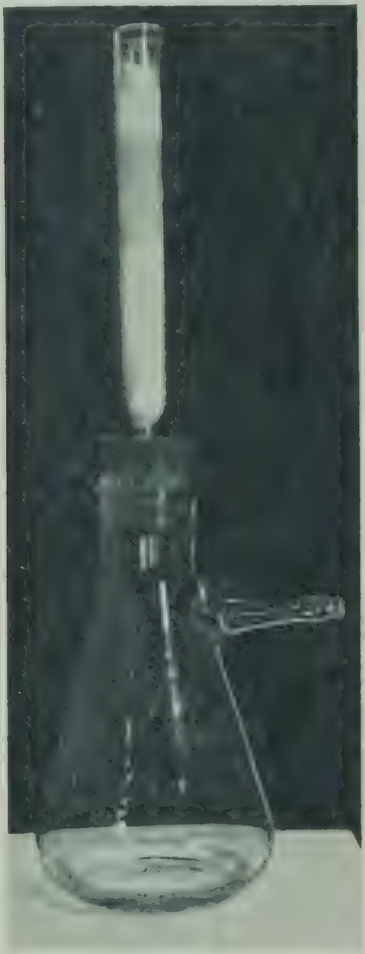

Fig. 83.—The Tswett apparatus.

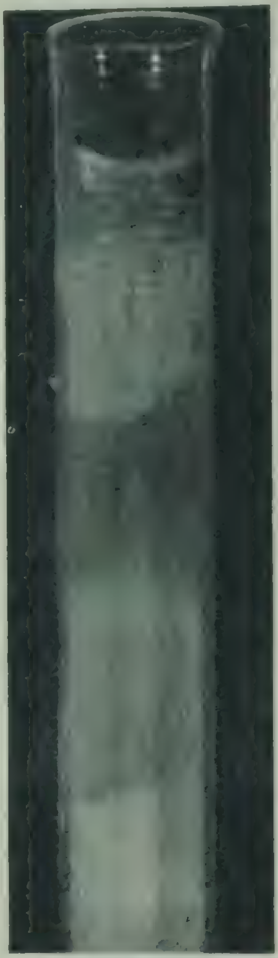

Fig. 84.—Chromatogram obtained with the Tswett apparatus.

1. Colourless.
2. Yellow = Xanthophyll B.
3. Olive-green = Chlorophyll B.
4. Blue-green = Chlorophyll A.
5. Yellow = Two mixed Xanthophylls.
6. Colourless.
7. Orange-yellow = Xanthophyll A.

Carotin is not adsorbed and comes through with the filtrate.

H. ETIOLATION. (See also Part IX, p. 433.)

Extreme etiolation can often be seen in Potato shoots which have sprouted from tubers in storage, where the weak colourless sprouts may be several feet long.

To obtain good etiolated seedlings it is desirable to use large seeds with considerable food reserves, such as various Beans or Peas. Sow in the usual way in two pots, placing one in the light and the other in a completely dark room. It has been shown that even five minutes daily exposure to light has a considerable effect in masking etiolation, so that care is needed to ensure that the room is completely dark and that there is no undue exposure to light when seedlings are examined and watered.

Compare the two sets of seedlings after about a fortnight. Observe that the etiolated shoots have greatly elongated internodes, colourless weak stems and small undeveloped leaves which are bright yellow with the colouring matter **etiolin.** This rapidly changes to Chlorophyll on exposure to diffused light. Strong light applied at once will kill the etiolated tissue. The apex of the stem often retains its embryonic hooked curvature instead of straightening up as do those in the light.

Cut sections of internodes of equal age from each type of seedling and note that in the etiolated stem the amount of vascular tissue is reduced and that there is more undifferentiated cambium than in the normal, while all the cell walls are thinner and the cells somewhat larger (see Part VIII, p. 390).

J. NITROGEN ASSIMILATION. PRESENCE OF NITRATES AND NITRITES IN TISSUES

1. The presence of nitrates in the tissues is tested for with Diphenylamine sulphate. In leaves the nitrate exists mostly as Calcium nitrate, and this is decomposed in light, the Calcium being deposited as Calcium oxalate and the Nitric acid assimilated. This only takes place in the presence of Chlorophyll. Cut thick sections of the leaves and mount in a drop of a 1 per cent. solution of Diphenylamine in 50 per cent. Sulphuric acid. A deep blue colour is an indication of nitrate. It is sometimes preferable to dry the section on the slide and use a solution made up in concentrated acid. This ensures a good colour where only small amounts of nitrate are present.

Good material to use is *Sambucus nigra* (Elder). Compare " sun leaves " and " shade leaves," also test sections of the leaf of the variegated Elder. The leaves, if they give a positive reaction, are placed in water in a bright light for several days, after which the nitrate will only be found in the colourless tissue. Compare also sections of the stems of Potato, Nettle and Sunflower taken at different levels. There is a marked difference between the amounts at the base and at the summit of the stem.

Potted plants of the Zonal Pelargonium with white-margined leaves may be used similarly. Place one plant in bright light and the other in deep shade and test after a spell of sunny weather. Plants in highly manured soil and plants in autumn usually contain much nitrate.

2. Although many plants possess the power of reducing nitrates, yet free nitrite is rare in plants and may be met with most certainly in etiolated shoots of Potato or in etiolated seedling Peas. The root tubercules of the Papilionaceae are also recommended. The juice expressed from the green shoots of Potato, Elm, Jerusalem Artichoke, Mignonette or *Solanum dulcamara* (Bitter Sweet) will, however, give good reactions as follows.

Express a few drops of juice and filter, if necessary, to remove Chlorophyll. Mix 3 drops on a slide with 1 drop of a 0·5 per cent. solution of Sulphanilic acid and 1 drop of glacial Acetic acid. Cool. Then add 1 drop of a 0·1 per cent. solution of *a*-Naphthylamine in water. A raspberry-red colour is positive.

K. TRANSLOCATION

1. Remove some leaves from a plant, *e.g.*, Tobacco or Pelargonium, in the late afternoon of a bright day, kill in boiling water and place in Alcohol over night. Next morning remove more leaves from the same plant and decolorize them in the same way. Test both for Starch. The Starch formed during the day has largely disappeared overnight.

2. Take some leaves from a plant at the end of a bright day and place their stalks in water. Cut out some pieces of the leaves at once and test for Starch. Repeat the test on these severed leaves the next morning. Has the Starch disappeared under these conditions ?

3. Place young plants of Broad Beans or Peas in darkness for a couple of days and then expose to sunlight for several hours. Gather and dry the green parts of the plants in the oven at about 100° C. Grind up the dry tissue and steep it in a little warm water for about half an hour. Filter.

Try the effect of this extract on a suspension of Starch in water, mixing about 2 c.c. of the extract with 10 c.c. of well-diluted Starch in a test-tube. Keep in a warm place and test a sample drop of the Starch periodically with Iodine. When the Starch reaction has practically disappeared, add equal amounts of Fehling's solutions I and II to the solution in the test-tube and boil. It will appear that the Starch has been converted into reducing sugar (a mixture of Maltose and Glucose). This is due to the hydrolysis of the Starch by diastase (amylase) in the leaves ; see next section.

Section 5. Enzymes

A. ACTION OF AMYLASE ON STARCH

1. Pound up 2 or 3 gm. of germinating Barley seeds or of commercial malt grains or of germinating seeds of Pea or Bean in a mortar and extract the mass with 50 c.c. of water. Filter and place two equal portions of the

filtrate in two test-tubes. Boil one tube and cool. To both tubes add an equal quantity of a dilute starch solution. Place both tubes in a beaker of water kept warm at 35° to 40° C. over a water bath. From time to time withdraw a small portion of each solution with a pipette and test each with Iodine solution in a watch-glass.

What is the reaction of the malt amylase to heating? Finally, test the remaining liquid in each tube with Fehling's solution. Which gives a reduction on boiling? To what is this due? (See p. 342.)

2. The above experiment may also be made to illustrate the effect of *p*H on enzyme activity. Make an extract of germinating or of malted Barley, as above, and make up a Starch solution with 2·5 gm. of soluble Starch boiled in 500 c.c. of 0·3 per cent. Sodium chloride.

Place 50 c.c. of the Starch solution in each of six small flasks and to each add phosphate buffer solutions as follows [1]:—

Flask number	1	2	3	4	5	6
0·1M Sodium dihydrogen phosphate, c.c.	10	8	6	4	2	0
0·1M Sodium monohydrogen phosphate, c.c.	0	2	4	6	8	10
Resultant *p*H	4·0	6·0	6·5	6·75	7·2	9·0

To each flask now add 5 c.c. of the amylase extract. From flask 4 remove a drop at short intervals and test it with a drop of Iodine. The original blue colour will gradually turn red, then yellowish. At this point take drops from all the other flasks in turn and test them with Iodine. It will be found that in flasks 1 and 6 there has been little or no change, and that the others show intermediate stages, flask 4 showing the maximum change. The sugar formed in this flask can be easily detected by taste.

3. *The Effect of Heat on Enzyme Action.*—Pound about 3 gm. of germinating or malted Barley in a mortar and extract the mass with about 50 c.c. of water. Filter through muslin. Into eight test-tubes place 10 c.c. of Starch solution and add 1 c.c. of the extract. Four of these tubes should be boiled. Place one boiled and one unboiled tube in water baths working at 20°, 25°, 30° and 35° C. Take the time for the Starch to be completely hydrolysed in each case. This can be done by taking out a drop of the solution at frequent intervals, placing it on a white tile and adding a drop of Iodine. So long as Starch is present the blue colour will be formed. From your results prepare a graph showing the relation between temperature and the time for the amylase to convert the Starch present completely into Maltose. Note that the enzyme is killed by boiling in each case.

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[1] Mixtures of N/10 Sodium hydroxide and N/15 Phosphoric acid may be used instead of the above.

B. ACTION OF LIPASE ON FAT

1. Shake up some Linseed oil with an equal volume of 50 per cent. Alcohol and test it with a litmus paper. The reaction is neutral. Take some *Ricinus* seeds which are beginning to germinate, chop and crush them up and add them to the oil mixture, shaking vigorously. Test again with litmus after a few hours. There is a marked acidity due to the formation of free fatty acid.

Test a small portion of the liquid for Glycerine. Add a little Copper sulphate solution and a few drops of Potassium hydroxide solution. A deep blue coloration is produced, but there is no blue precipitate.

2. A quantitative estimation of lipase activity may be easily made by titration. One gram of *Ricinus* seeds, freed from shell, is ground up in 25 c.c. of Chloroform water. Place 10 c.c. of the suspension in each of two test-tubes and add 1 c.c. of dilute Acetic acid (about 0·5 per cent.) to liberate the enzyme. Boil one tube and place both in a water bath at about 40° C. for an hour. The oil in the seeds is acted upon by the lipase of the seed itself. Add a few drops of Phenolphthalein as indicator and titrate with 0·1N alkali. More will be required for the unboiled tube, showing the formation of free fatty acid in addition to the Acetic acid originally present. In the boiled tube the enzyme has been destroyed.

C. ACTION OF PROTEASES ON PROTEINS

In this case the reactions are best tested with commercial preparations of the enzymes. Pepsin and Papain may both be obtained in solid form and are fairly stable. The Erepsin type of protease (Trypsin) is commoner in plants and may be obtained from raw material such as Hemp seeds.

1. *Pepsin and Papain.*—Place some dried blood fibrin in a solution of Congo Red until it is deeply stained (twenty-four hours). Filter off the fibrin and wash thoroughly on the filter in running water. Squeeze out excess of water. The stained fibrin may be preserved in glycerine.

Place 5 c.c. of an aqueous solution of the enzyme in a test-tube with 5 c.c. of 0·04 per cent. Hydrochloric acid and add a pinch of the stained fibrin. Keep at about 40° C. in a water bath for an hour. The colour is released by the digested fibrin, and is blue in acid solution. Add excess of solid Sodium carbonate to change the colour to red and to stop the action. Compare the action of equal amounts of Pepsin and Papain in the above way. To the deeper coloured of the two extracts add water from a burette until it matches the other colour. The relative activities of the enzymes are proportional to the final volumes of the adjusted extracts.

2. Stir egg-white in a basin until it is uniform in consistency, then slowly mix with it four times its own volume of tap water. Heat in a water bath at 60° C. for twenty minutes, then strain through muslin. This gives a cloudy suspension of coagulated albumen. Dilute this, one to nine, with tap water before use. Place 5 c.c. in a test-tube and add 5 c.c. of 0·4 per cent. Hydrochloric acid. Add also about 1 c.c. of Pepsin solution and keep warm

for about half an hour. The cloudy liquid will become quite clear as the albumen is digested.

Two enzymes may also be compared by this method by making up a series of tubes of albumen suspension and adding graduated amounts of enzyme to each. The ratio of the concentrations which show complete clearing in thirty minutes, gives the relative activities of the enzymes.

3. *Erepsin.*—Commercial Trypsin may be used, or seeds, leaves or roots may be tested by the general method below.

Pound up a few grams of the fresh tissue or seeds employed, *e.g.*, Beans, Peas, Hemp seeds, Carrot, Cabbage, etc., with 100 c.c. of water containing a few drops of Toluol as a preservative.

Filter, then add a small quantity of Peptone on a knife point and shake up. Keep in a warm place for two to three days, then test as below for the amino-acid Tryptophane, which is easily distinguished. Filter the extract and acidify with a few drops of dilute Acetic acid. Add Bromine water carefully, drop by drop, until a purple colour develops, indicating Tryptophane. In case of doubt shake up with a little Amyl alcohol. On standing, this will rise to the top, carrying with it any of the purple colour formed. Test for unaltered Peptone with the Biuret Test. To a few cubic centimetres of the solution add about 1 c.c. of 40 to 50 per cent. Sodium hydroxide and 1 drop of a dilute Copper sulphate solution. Proteins and Peptones give an intense purple colour.

4. *Protease Activity in Relation to p*H.—Experiment 1 above may be modified to provide a test of the difference between Pepsin and Trypsin in regard to the most favourable reaction for their proteolytic activity.

Take four tubes. Into two of them place 5 c.c. of 0·04 per cent. Hydrochloric acid and in the other two place 5 c.c. of 0·04 per cent. Sodium hydroxide. These solutions are approximately M/100 in each case. To each tube add a pinch of stained Fibrin and as much solid commercial enzyme preparation as can be taken on the point of a penknife. Add each enzyme to one acid and one alkaline tube respectively. Shake well to mix with the liquid and place in an incubator or water bath at 37° C. for some hours. In the tubes, when digestion of the Fibrin takes place, the Congo Red will be released into solution, and the depth of colour of the liquid gives a measure of the amount of digestion which has occurred. Note that Congo Red is blue in acid solutions. To facilitate comparison it is therefore better to add excess of alkali to all tubes at the end of the experiment.

D. OXIDISING ENZYMES

Two chief types of enzyme are included under this term, oxidase and peroxidase, both catalysing coupled oxidation-reduction processes. The first, oxidase, is a catalyst which brings about the reduction of molecular Oxygen with the simultaneous oxidation of some other substance. Its action is independent of the presence of Hydrogen peroxide. It reduces only Oxygen, not dye-stuffs, and in this differs from dehydrogenase.

The second, peroxidase, acts only upon Hydrogen peroxide and catalyses oxidations by the atomic Oxygen thus liberated.

In plants the principal oxidase system is one which oxidises phenolic substances in the presence of Oxygen. For this reason it has become known as the polyphenol oxidase or oxygenase. According to Onslow, oxidizable phenols with the accompanying enzyme are found in about 63 per cent. of higher plants.

1. Cut open the surface of a Potato tuber and allow the cut surface to dry up or blot it with filter paper. Place on the dry surface a drop or two of a freshly made solution of Guaiacum gum [1] in absolute Alcohol. A deep blue colour develops.

Guaiacum gum contains Guaiaconic acid, one of the most sensitive reagents for oxidation. It forms Guaiacum blue. It is used for preference where only small amounts of active oxygen are available, as in the above experiment.

2. The polyphenol oxidase will also oxidise Dimethyl *p*-phenylene diamine to Indophenol blue in the presence of an oxidizable phenol, *e.g.*, α-Naphthol or Catechol. The phenol is oxidized to an orthoquinone, which then acts on the diamine.

To the extract of Potato tuber above add a small quantity of the following mixture (Nadi reagent) :—

A	α-Naphthol (0·14 per cent. solution)	5 c.c.
	Dimethyl *p* phenylenediamine (0·17 per cent. solution) .	5 c.c.
B	Sodium carbonate (0·1 per cent. solution)	10 c.c.

Mix solutions A and B immediately before use. A dark-blue colour shows the presence of the oxidase, but the colour must be immediately produced. Catechol oxidises slowly but spontaneously in air without the presence of the enzyme, so that it will eventually react with the diamine and produce Indophenol blue, especially if there are traces of heavy metals present. The presence of the oxidase greatly hastens the action.

3. The presence of peroxidase may be shown in the ground-up tissues of the Horse Radish. Prepare as for the oxidase test and add a little Guaiacum solution. No blue colour appears until a few drops of Hydrogen peroxide are added.

4. A third enzyme, catalase, is usually present in plant tissues, which is also concerned with Hydrogen peroxide, though it does not produce oxidations, as the Oxygen it releases is in the molecular form. Its function is uncertain, but it certainly will protect cells against the accumulation of Hydrogen peroxide.

As peroxidase and catalase are both thermolabile and are inhibited by the same substances, we can only demonstrate the presence of catalase by the formation of gaseous Oxygen from Hydrogen peroxide.

Fill a test-tube with Hydrogen peroxide (20 vols.) diluted with an equal amount of water and add about 1 gm. of Potato tissue which has been ground up thoroughly under Alcohol and filtered with a filter pump until dry. Invert the mouth of the tube into water in a basin. A rapid evolution of gas takes place. On testing this will be found to re-ignite a glowing splinter. Boiled tissue will not produce this effect as the enzyme is destroyed.

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[1] It is advisable to break open a lump of the gum and make a solution only from the inner portions.

5. The action of a dehydrogenase in promoting oxidation may be shown practically by the use of Methylene Blue as an acceptor for the Hydrogen. The blue solution is thereby reduced to a colourless state. Place 1 c.c. of a 15 per cent. solution of Glucose in a test-tube. Add 1 c.c. of a suspension of either living yeast or of zymin (see p. 358) and fill up the tube with a very dilute solution of Methylene Blue of a light colour. Cork carefully, excluding all air, and place for a short time in a warm place. The oxidisation of the Glucose is accompanied by the reduction of the Methylene Blue to its leuco-base.

Section 6. Respiration

A. AEROBIC RESPIRATION

1. A very simple titrimetric method is recommended by Boysen Jensen. A wide-mouthed tube about 10 cm. by 4 cm., or a bottle of corresponding size, is fitted with a waxed cork, and a stand to cover the bottom is made by bending down the corners of a square of perforated zinc to act as feet. In the bottom of the tube is placed exactly 2 c.c. of N/22 Barium hydroxide. The stand is next placed in the tube and a few leaves of, say, Oak or Beech, which nearly fill the tube, are placed on the stand clear of the baryta. The area of these leaves should be measured (see p. 315). Cork the tube and stand it in a warm place in the dark for about three hours. The temperature should remain as constant as possible. Remove the cork, leaves and stand, and immediately titrate the Barium hydroxide with Phenolphthalein and N/110 Hydrochloric acid. A blank experiment should also be set up, as before, but without leaves or stand, but containing the same amount of baryta, which is titrated after the lapse of the same period of time. The calculation should express the Carbon dioxide evolved in milligrams per 50 sq. cm. of leaf per hour. In a given case, where 30 sq. cm. of leaf was used and the experiment lasted for three hours (180 minutes), it was found that the baryta needed 4·1 c.c. of the Hydrochloric acid to neutralize it. The blank required 9·6 c.c. of acid.

Then:—

$$\frac{(9{\cdot}6-4{\cdot}1)\times 0{\cdot}2\times 50\times 60}{30\times 180}=0{\cdot}61 \text{ mg. of } CO_2 \text{ per 50 sq. cm. per hour.}$$

1 c.c. of N/110 Hydrochloric acid is equivalent to 0·2 mg. of Carbon dioxide.

2. Another very simple method is described by Neilson Jones. In this case the evolution of Carbon dioxide is measured by the change in colour of an indicator solution.

Take about 1 litre of distilled water and add a few drops of an alcoholic solution of Brom-cresol-purple (0·04 per cent.), so that when the liquid is examined in a wide test-tube there is a distinct but pale colour. Divide the solution into two parts. To one add a few drops of lime water and to the other a few drops of a phosphate buffer solution, *i.e.*, Sorensen's Na_2HPO_4, 2 parts, with KH_2PO_4, 8 parts (see Appendix), giving a *p*H of 6·2. Into two large test-tubes about 1 in. in diameter pour enough of each solution to make a layer about 1 in. deep. Cork the one containing the solution with the phosphate buffer and keep it as a standard. The other is also corked, and is used for the experiment. Make a little cage of perforated zinc, which can be hung from the cork inside the test-tube. It should be long enough to hold about six Peas in a line. Fill it with soaked Peas and hang it inside the tube containing the indicator and lime water. After about half an hour at laboratory temperature enough Carbon dioxide will have been produced to form Calcium bicarbonate in the liquid. This turns the indicator yellow. By blowing ordinary air through the liquid with a bellows this Carbon dioxide can be driven off again and the liquid turned back to purple. The experiment may be repeated as often as desired to obtain a good average. The reciprocal of the time taken for the purple indicator to arrive at a colour which matches that in the standard tube (*p*H 6·2) is a measure of the intensity of respiration. By this method the effect of temperature, anaesthetics, etc., can be easily shown. If a green leaf is substituted for the Peas it will be found that the solution changes from purple to yellow in the dark, but turns back again to purple when brightly illuminated, showing that the leaf reabsorbs the Carbon dioxide for photosynthesis.

B. RESPIRATION OF STARCHY AND FATTY SEEDS

1. A useful apparatus for respiration studies on seeds is McDougall's Respiroscope. This is shown in Fig. 85. A little damped glass wool is placed at the bottom of the wide tube and on this a measured weight of soaked seeds is placed. The small inner tube contains a strong solution of Sodium or Potassium hydroxide to absorb the Carbon dioxide formed in respiration, and the long narrow tube dips at the bottom into Mercury, or, alternatively, Glycerine coloured with a dye. The rubber cork is pierced by a piece of glass tubing to allow the escape of air as the cork is pushed home. This has a piece of rubber tubing attached to it, which is closed by a clip when the cork is in place, and for additional security a piece of glass rod is pushed into the free end of the rubber tube as a bung.

The Carbon dioxide produced by the respiration of the germinating seeds is absorbed by the alkali in the small tube, and the pressure deficit thus caused draws liquid up the stem tube.

While essentially a qualitative apparatus, it can be used to give semi-quantitative results by carefully measuring the area of the bore of the tube, so that the volume of the liquid drawn up in unit time can be calculated. Irregularities in the bore may be ignored for this purpose.

In this way a comparison may be made of the respiration of starchy seeds such as Barley or Oats and of fatty seeds such as Linseed. The fatty seeds will be found to give an apparently higher rate, because of the Oxygen absorbed for the oxidation of fatty reserves, in addition to that used for true respiration. In other words, in this case the ratio of $\frac{O_2}{CO_2}$ is greater than unity.

On the other hand, if no alkali is included in the apparatus, the starchy seed should show no rise of liquid, the respiratory quotient being unity. The fatty seed on the other hand will show a rise of the liquid, though less than before, due to the excess of Oxygen absorbed.

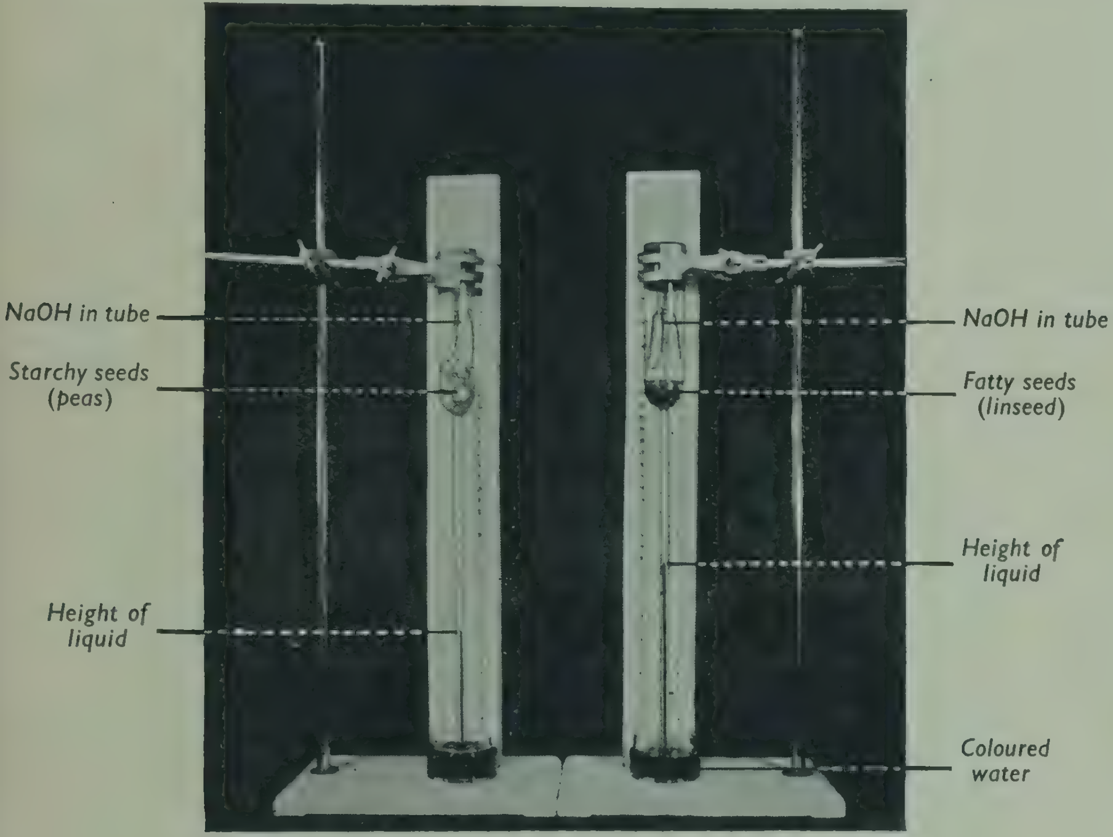

FIG. 85.—The McDougall respiroscope, for the comparison of respiration in starchy and fatty seeds.

2. *Ganong Respirometer.*—The apparatus is illustrated in Fig. 86. There are three parts. First, the bulb for the material, with a small water-bulb at the bottom and with a stopper provided with a lateral hole which can be closed or opened by turning the stopper. Secondly, the measuring cylinder opening out of the bulb ; and thirdly, the reservoir or balancing cylinder connected to the first cylinder by stout rubber tubing. Oat seeds are started in a germinator and ten are selected when the roots begin to show and placed in the bulb. These seeds occupy practically 1 c.c., and 1 c.c. of water is added. These 2 c.c. subtracted from its volume leave 100 c.c. of air in the

bulb. Pour the index liquid (see below) into the reservoir tube until it stands at the 100 mark in the graduated cylinder and at the upper mark in the

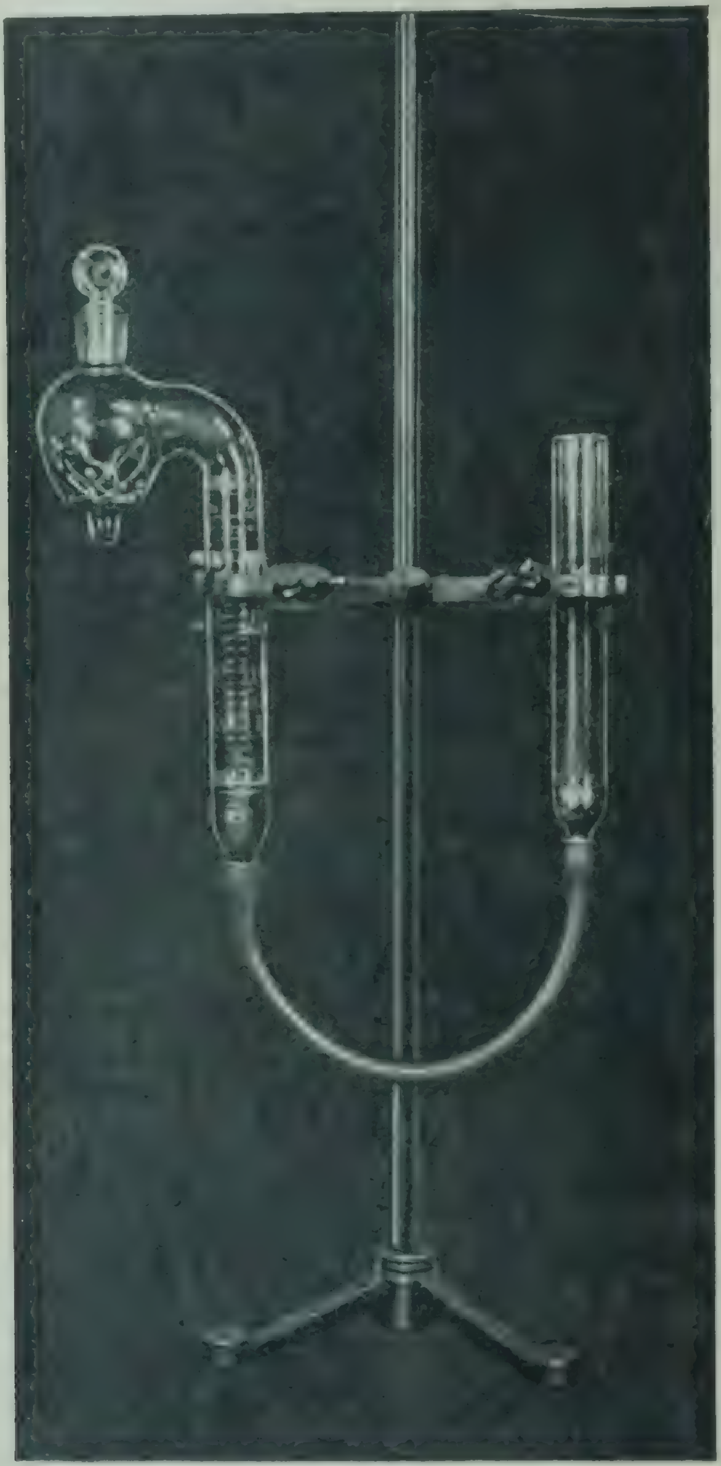

FIG. 86.—The Ganong respirometer.

reservoir (*lower* mark with Mercury). Oil the stopper and insert it into the bulb. Place the hole at the open position to release the air pressure inside, then twist to close. If strong alkali (soda or potash) is used as an index liquid it will rise in the course of the experiment nearly to the 21 mark and stop, showing that all the oxygen has been converted into Carbon dioxide. Wash out the alkali, start with a fresh lot of seeds and fill the reservoir tube with Mercury. This maintains a constant level, showing that the ratio of Oxygen absorbed to Carbon dioxide emitted is unity. A third experiment may be made using a strong mixture of Pyrogallol and Sodium hydroxide in the reservoir. This will promptly absorb all the oxygen in the bulb. The seeds thereafter fail to develop, showing that the gas absorbed by them in normal respiration is Oxygen, which is replaced by an equivalent volume of Carbon dioxide emitted.

In measuring all gas volumes the levels in the reservoir and the graduated cylinder must be equalized by sliding the reservoir up or down. The temperature should be read at the beginning and end of the experiment, and, if different, any consequent changes in the gas volume allowed for. For very exact readings allow also for vapour pressure and calculate the gas volume to Normal Temperature and Pressure.

C. QUANTITATIVE MEASUREMENT OF RESPIRATION

1. A more exact respiration measurement can be made by means of a "gas-train" arrangement, whereby the Carbon dioxide is absorbed in Barium hydroxide solution, the amount absorbed being measured by titration with dilute Hydrochloric acid.

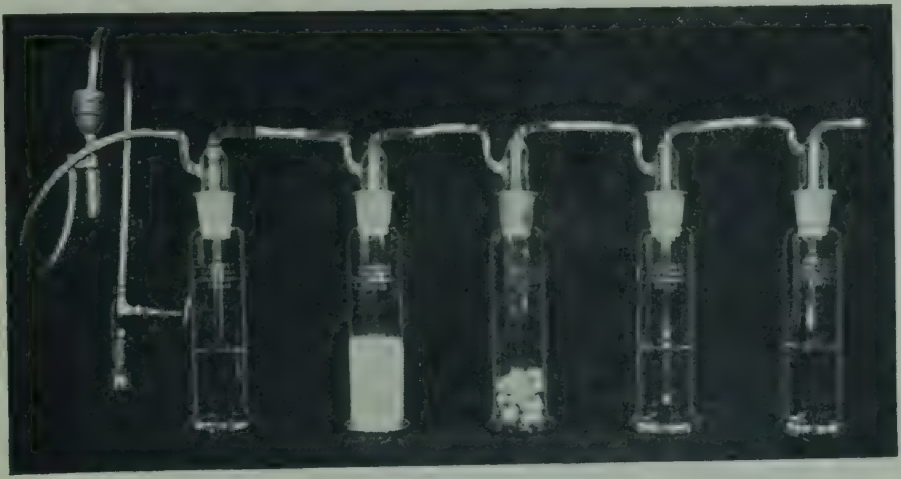

Fig. 87.—Gas-train to measure the carbon dioxide evolved by seeds during respiration.

Connect five gas-washing bottles together with thick-walled rubber tubing as shown in Fig. 87. Each bottle should hold about 250 c.c. It is not necessary that they should have blown-glass stoppers, though this makes the fitting-up easier and avoids all possibility of leaks. Bottles fitted with good rubber corks and carefully bored to take a couple of glass leading tubes through each cork give perfectly good results if well fitted and if each cork is luted with Vaseline after setting up.

The connections must be made as shown, *i.e.* (counting from the right), short limb to long limb. Bottle 5 is attached to an aspirator or water pump by the short limb, to draw a current of air slowly through the train of bottles.

Bottle 1 contains a strong (20 per cent.) solution of Potassium hydroxide to absorb the Carbon dioxide from the entering air. Bottle 2 contains Barium hydroxide solution as a " tell-tale " to show whether the absorption in Bottle 1 is complete. It should remain clear throughout the experiment. If any white precipitate appears in it the experiment should be started again, using a stronger solution in Bottle 1 and, if possible, reducing the rate of suction of air through the apparatus.

In Bottle 3 place about fifty germinating Peas lying on a pad of wetted glass wool. Bottle 4 contains about 150 c.c. of decinormal Barium hydroxide, this time of a solution standardised against decinormal Hydrochloric acid, using Phenolphthalein as indicator. The end point is taken as the last perceptible trace of pink coloration, when the acid is run into the baryta solution and the sample should be retained as a standard of comparison in the titration after the experiment. Bottle 5 contains more Barium hydroxide as a " tell-tale " to show if the absorption of Carbon dioxide in Bottle 4 is complete.

Set the air current going slowly with the water pump. The Carbon dioxide formed by the respiration of the Peas will cause a white precipitate of Barium carbonate in Bottle 4. An estimate of the amount of Carbon dioxide absorbed may be made at the end of an hour. Stop the flow of air and withdraw 20 c.c. of baryta solution from Bottle 4. Run this gently into a small beaker and titrate with N/10 Hydrochloric acid as above. It does not matter if the sample is cloudy as the dilute acid does not attack Barium carbonate. The apparatus may be re-started at once and further estimates made at equal intervals for three more trials. After that it is best to refill Bottle 4 with fresh baryta if further samples are desired.

The equivalence is given by the factor :—

$$1 \text{ c.c. of N/10 HCl} = 0{\cdot}0022 \text{ gm.} CO_2 = 1{\cdot}11 \text{ c.c. at N.T.P.}$$

2. Interesting comparisons may be made quantitatively between the respiration rates of different plant organs by the above method.

Compare especially germinating seeds and opening leaf buds ; leaves in darkness and in light of different intensities (look for the " compensation point " at which the rate of photosynthesis exactly balances the respiration rate) ; floral parts, comparing petals with both types of sex organs ; and lastly leaves (in darkness) with roots. Seasonal differences due to age may also be discovered in one type of organ. A good many lines of research readily suggest themselves to those who have time to follow up these experiments.

3. *Simplified Barcroft Method* (Thoday).—The apparatus is illustrated in Fig. 88. It can be easily constructed from ordinary laboratory materials. A is the experimental chamber. The weighed respiring material (2 to 3 gm.), enclosed in muslin or wire gauze, is suspended in A from a hook under the rubber stopper. B is a compensating chamber which acts as a control. The U-tube manometer C, which connects A and B contains a light lubricat-

ing oil. D is a graduated tube, such as a 10 c.c. pipette with the ends cut off, and E is a levelling reservoir sliding vertically in a spring clip and containing water. If Mercury is used in C, D and E, then D should be a 1 c.c. graduated pipette.

If the apparatus is mounted on a wooden block, as shown at B, the two chambers can be immersed in water in a trough, which will help to maintain an even temperature, while the manometer and the other tubes are outside, against the front of the trough.

Place equal volumes of 10 per cent. Potash in each chamber and suspend the respiring material in A. Do not handle A more than is essential, as this tends to warm the glass. Open the outlets to A and B and adjust the level of water in D to the lowest mark. Close the outlets and, by adjusting E, level the liquid in the manometer to the same height in both arms. A horizontal mark across both arms is a help in doing this. The outlet tubes should be fitted with pressure rubber-tubing, or glass taps may be fitted here as an improvement.

After the lapse of an experimental period, the oil in the manometer will have risen towards A. If it does not, there is probably an air leak somewhere. By raising E, bring back the oil to the same level in both arms of C. The change of volume read in D is a measure of the volume of CO_2 which has been absorbed by the Potash in A, and hence of the rate of respiration of the material. The best results are obtained by using a time period which gives a volume change of about 0·5 c.c. Fresh air may then be aspirated through A by means of the outlet tubes, and the experiment repeated several times to obtain an average.

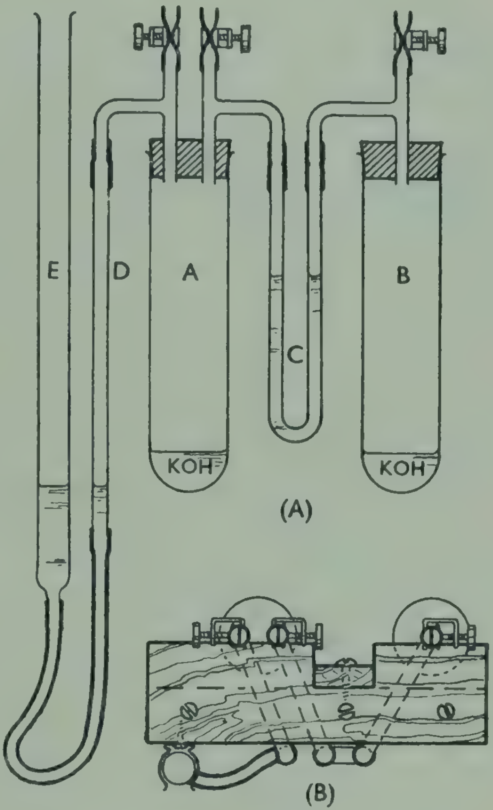

FIG. 88.—Thoday's pattern of simplified Barcroft apparatus.
(*Adapted from Thoday, "School Science Review," 54, 1932.*)

For a fuller description of the above method, see Thoday, *The School Science Review*, No. 54, December 1932.

D. EXCRETION OF CARBON DIOXIDE BY ROOTS: MICROSCOPIC OBSERVATIONS

1. Fix two small strips of glass to a microscope slide with Canada Balsam, leaving between them a channel about 5 mm. wide and 2 to 3 mm. deep.

Place the slide, when dry, on the microscope stage and fix a seedling of one of the cereal grains, either in a clamp, or pinned to a cork in such a position that one of the root-tips lies in the channel on the slide (Fig. 89). Choose a thin rootlet, so that a cover-glass may lie flat upon it, resting on the two glass strips. With a pipette run in a drop of aqueous solution of Bromthymol-blue into the channel so that the root is immersed in the blue solution.

The solution is made by grinding up 0·1 gm. of the indicator with 3·5 c.c. of N/20 Sodium hydroxide in a small mortar and diluting to 250 c.c. with water. This gives a solution of the blue Sodium salt of the dye which turns yellow with acids.

If the root is watched through a microscope a narrow yellow halo appears around it showing the diffusion of Hydrogen ions from the tissues. This halo becomes broadest at the meristematic apex and is narrowest round the older portion of the root, illustrating differences in their respective rates of metabolic activity.

That the Hydrogen ion concerned is in Carbonic acid may be shown by substituting a 0·01 per cent. solution of Congo Red in tap water for the Bromthymol-blue. Congo Red turns blue with most acids, but with Carbonic acid, which is an extremely weak acid, the red solution turns purple.

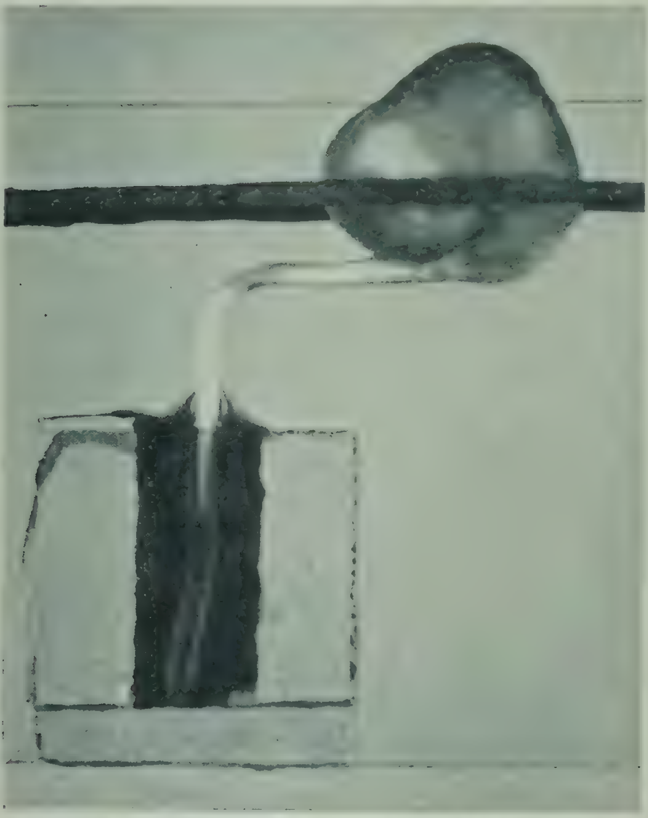

Fig. 89.—Apparatus to demonstrate the evolution of carbon dioxide by roots.

2. One of the classic experiments of plant physiology illustrates the excretion of Carbon dioxide from roots by its effect on a marble slab. A piece of marble is buried flat in soil and a seed of Broad Bean is planted above it. The root system will spread out over the marble and, in the course of a few weeks, will etch a perfect representation of itself by means of excreted Carbonic acid.

E. PRODUCTION OF HEAT BY RESPIRATION

In the plant most of the energy derived from respiration is utilized in growth and metabolism, but some appears as heat, as in warm-blooded animals. This is especially the case where reserves are being rapidly consumed.

1. Take two thermos flasks and partly fill one with fresh germinating

Peas. Into the other place a similar lot of Peas which have been killed by steeping overnight in 1:1000 Mercuric chloride solution. Carefully match two thermometers and place one in each flask. Note the difference in temperature after an hour.

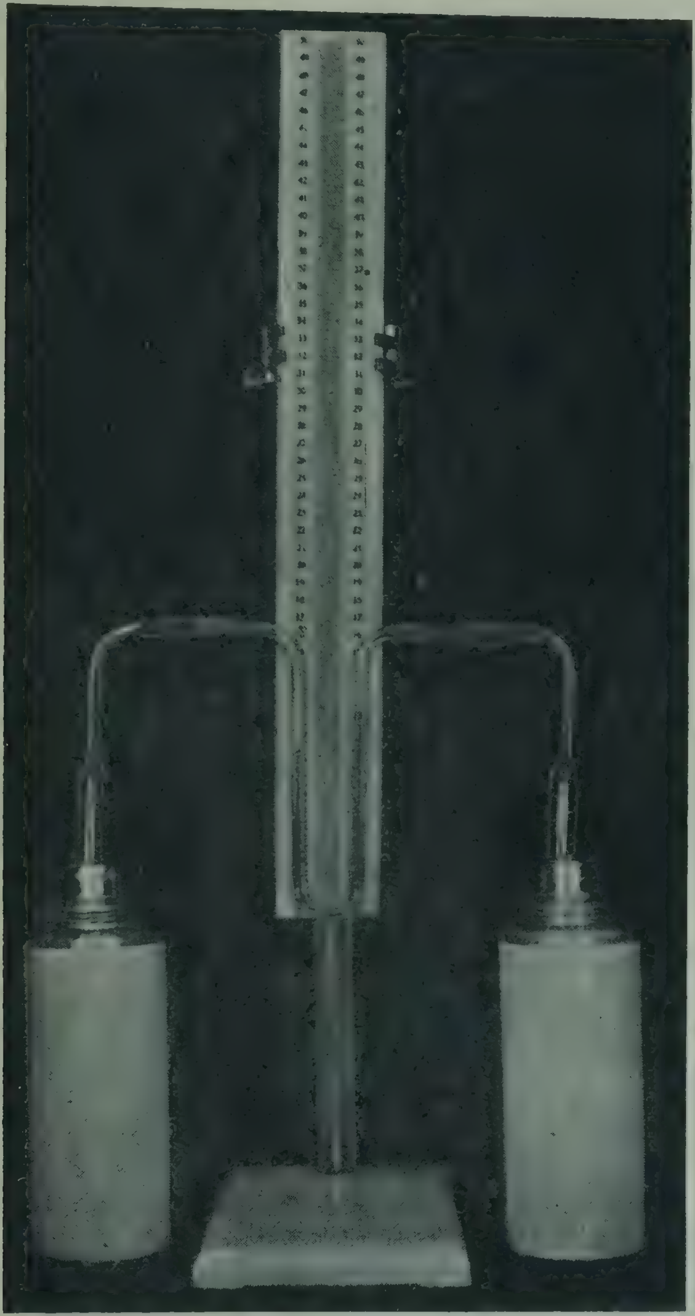

FIG. 90.—Apparatus to demonstrate the heat of respiration in seeds.

A more striking demonstration is obtained by using a differential air thermometer, as shown in Fig. 90. Two cylindrical glass bulbs are connected by a U-tube containing a coloured liquid, *e.g.*, liquid Paraffin coloured with Sudan III. One bulb is placed in each flask. The expansion of air on the side of the living Peas, due to the heat of respiration, drives the liquid in the U-tube well up the other side and will before long drive it right over into the other bulb.

2. Wounding increases the rate of respiration. Drop a handful of slices of peeled Potato into a beaker of water and note the marked rise in temperature which soon follows.

F. LOSS OF WEIGHT DUE TO RESPIRATION

In light, during normal growth, this loss is more or less compensated by photosynthesis. Nevertheless, it occurs and must be added to the " apparent assimilation " to find the " true assimilation " or gross gain in weight due to photosynthesis. In the dark, respiration losses are uncompensated and are therefore more noticeable.

Take about fifty Peas, selected for similarity in size. Pick out half a dozen and dry them thoroughly in an oven at about 100° C. Weigh and take the average weight. Sow half of the remainder in Coconut fibre or sawdust in a box kept in full light and the other half in a box kept in the dark.

At the end of each week measure the average height of the seedlings in the two boxes. Remove three or four from each box. Wash their roots perfectly clean and dry them in an oven at 100° C. When quite dry weigh them and record the average dry weight. After four weeks plot the results in four graphic curves : the heights and dry weights of the seedlings in the light and the heights and dry weights of the seedlings in the dark. All four may be on one sheet of graph paper if different colours or signs are used for each.

The results should show clearly that growth, in the sense of increase in size, is quite independent of the addition of solid weight, and may occur even when dry weight is decreasing.

G. ANAEROBIC RESPIRATION

That living organisms normally require a free supply of Oxygen scarcely needs demonstration, but many people are apt to overlook the fact that germinating seeds are in the same category, and that deep sowing is fatal. The restriction of respiration due to shortage of Oxygen deprives the seed of its main source of energy.

1. Prepare a wooden box with one glass side, or take a tall glass jar. Place an inch or two of damp soil in the bottom and sow a few soaked Peas close to the glass. Add more soil, inch by inch, sowing a few more seeds at each level, pressing the soil firmly down at each stage. Set aside in the dark and watch the behaviour of the planted seeds. Note the maximum depth at which germination takes place.

2. Even the higher plants have a limited power of respiration without Oxygen, but it is insufficient to maintain growth.

Soak three or four Peas for a couple of days and then carefully remove the skins so that no air may remain trapped inside. Fill a large test-tube with Mercury and invert into a Mercury trough so that it includes no air bubbles. Push the skinned Peas one by one under the rim of the tube and they will rise through the Mercury to the top (see Fig. 91). Put aside in a warm place. After a few days the Mercury will be seen to have been displaced downwards for some distance by an accumulation of gas round the Peas. Damp a fragment of Potassium hydroxide and push it under the tube. When it rises to the top it will absorb almost all the gas produced, showing that the gas was Carbon dioxide.

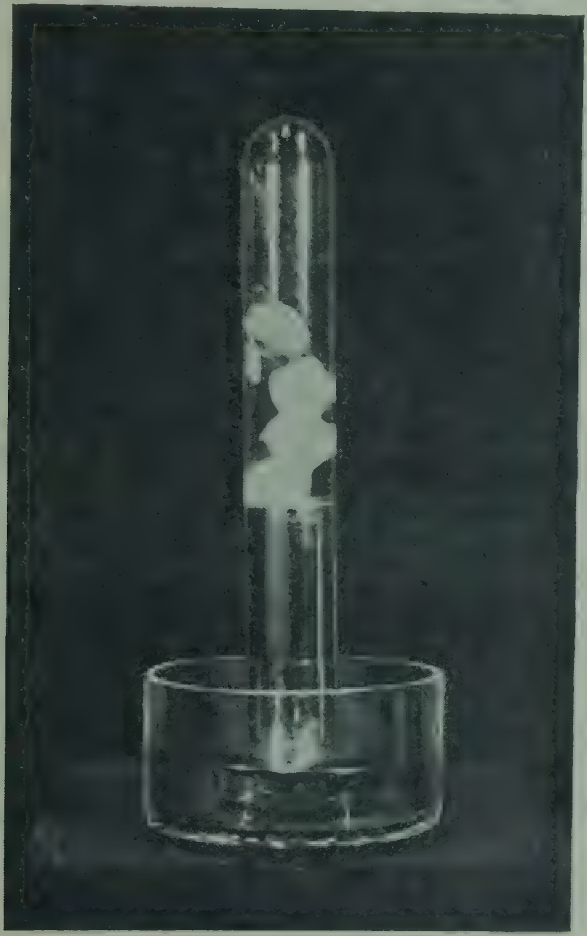

FIG. 91.—Apparatus to demonstrate anaerobic respiration.

This type of respiration without air (anaerobic) is characteristic of some lower organisms, principally among the Bacteria, some of which are obligate anaerobes, *i.e.*, are incapable of growing in the presence of Oxygen.

H. FERMENTATION OF SUGAR BY YEAST

Ordinary bakers' yeast may be used or, if convenient, beer yeast may be obtained from a brewery. Both types are strains of *Saccharomyces cerevisiae*. Yeast will ferment either Dextrose (Glucose) or Sucrose, but it first hydrolyses the latter, so it is preferable to start with Dextrose.

1. Make up a 15 per cent. solution of Dextrose in water. Place about 250 c.c. in a flask fitted with a leading tube bent twice at right angles (see Fig. 92) and dipping into a solution of Barium hydroxide contained in a second corked flask. A tube from the second flask leads into a third flask containing 10 per cent. Sodium hydroxide as a trap to prevent interference by atmospheric Carbon dioxide.

Place the apparatus in a warm place. Fermentation will soon begin, and the evolved Carbon dioxide bubbles over into the baryta water and produces a white precipitate of Barium carbonate. Notice that in the early stages the fermenting liquid froths freely and that the yeast is mostly at or near the surface. After a day or two, however, when the sugar has been nearly all fermented, the froth subsides and the liquid begins to clear. The yeast cells, which are now loaded with Glycogen reserves, sink to the bottom. Uncork the fermentation flask. The smell of Alcohol will probably be easily detected, but its presence may be proved chemically by the Iodoform test. Decant a few cubic centimetres into a test-tube and heat gently. Add gradually a strong coloured solution of Iodine in Potassium hydroxide. A yellow crystalline precipitate forms and the characteristic penetrating smell of Iodoform develops. Examine a drop of the liquid under the microscope. The Iodoform appears as yellow hexagonal plates.

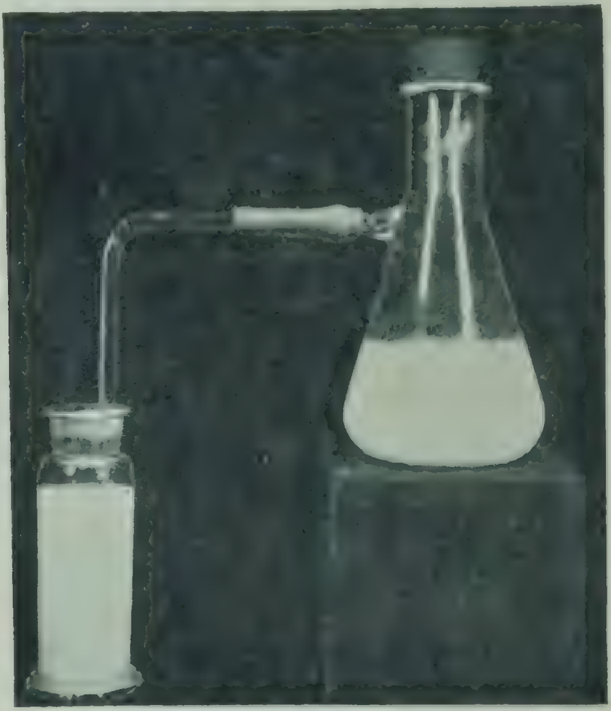

FIG. 92.—Apparatus to demonstrate the fermentation of yeast.

2. The separation of certain constituents of the zymase in killed yeast may be accomplished by dialysis. The specially killed yeast, known as "zymin," is prepared as follows: Wash the fresh yeast several times with distilled water and filter it off with a filter pump. Weigh out 5 gm. of the

washed yeast and stir it up well in a mixture of 60 c.c. of absolute Alcohol and 20 c.c. of Ether for four to five minutes. Filter off and wash on the filter with a few cubic centimetres of Ether. Spread out and allow to dry. Prepare a collodion sac on a wide test-tube as a mould (see p. 302). Into the sac pour a 30 per cent. solution of Dextrose mixed with about 2 gm. of zymin. Immerse the sac, up to the top, in a jar of distilled water which should be continuously or at least frequently stirred. Fermentation will begin in the sac, but will soon slow down and stop. More sugar may be added, but no further action will take place until a little of the water from the jar is added to the liquid inside the sac. The dialysable substance is not the enzyme itself, since it will not by itself start fermentation in a fresh lot of sugar solution. Furthermore, its properties are not destroyed, as are those of the enzyme, by boiling. In fact two substances have dialysed from the sac: one is the co-zymase, which is probably Adenosin, derived from Nucleic acid, and the other is a phosphate which forms a compound with the sugar, being fermented through a linkage with the co-zymase.

Section 7. Growth

A. GERMINATION OF SEEDS

In Part V, Section 5, page 260, a number of types of seed germination are described. This is the commencement of the plant's growth as an independent individual. What are the conditions necessary to start growth ? That Oxygen is needed has been shown on page 356 by an experiment on the depth of sowing, where it was pointed out that the reduction of Oxygen supply restricts respiration, so that seeds are deprived of their source of energy.

1. The same effect is shown if two lots of Peas are placed in two large beakers of water. Let one stand, and place the other under a slowly running tap, so that the water splashes in the beaker as it falls. In the first case the seeds rapidly decay, but in the second, where the water is continually aerated, they remain healthy and germinate.

2. The need for an adequate temperature is sufficiently shown by the fact that seeds do not as a rule germinate in winter, and certainly not during the coldest weather. The need for water is also universally demonstrated by the dry seeds of commerce, which remain dormant for long periods, even sometimes for many years, if not wetted. These " dry " seeds do, in fact, contain a small percentage of water, which is essential for their continued existence, though not enough for growth. Chop up a dry Pea and drop it into a test-tube. If the tube be now cautiously warmed over a Bunsen burner, drops of water will collect on the inside of the tube near the mouth, which have been driven off from the seed. Such seeds usually contain water to about 10 per cent. of their dry weight.

3. Light affects the germination of only a few seeds. Some are negatively

light sensitive, *i.e.*, will not germinate if exposed to light after having imbibed water. Such are *Nigella sativa, Datura stramonium* and *Phacelia tanacetifolia*. On the other hand, seeds of *Nicotiana, Digitalis, Daucus carota* and *Verbascum thapsus* are all light-stimulated and germinate little, if at all, in darkness. The effect is much more marked with fresh seeds than with those which have been stored. This light sensitivity, which may be due to an effect on the lipase enzymes, is not a general property of seeds, and it is not fully investigated. On the other hand light is, of course, essential for the development of all seedlings after germination has taken place.

B. DISTRIBUTION OF GROWTH IN ROOT, STEM AND LEAF

The unequal distribution of growth rates in young organs may be demonstrated by a system of marking.

1. Germinate some Broad Beans in damp Coconut fibre or else by pinning them to the underside of the cork in a glass jar partly filled with water. By the latter method previously well-soaked seeds will germinate to give beautifully clean roots, without touching the water or soil.

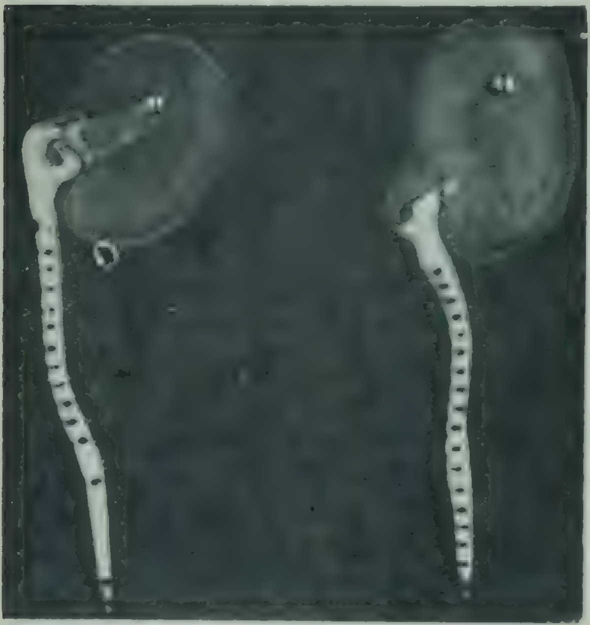

FIG. 93.—Method of marking Bean roots to demonstrate the region of active growth.

When the roots are at least 1 in. long (four to six days) they should be carefully marked with a series of transverse lines in Indian ink (Fig. 93). This may be done either with the milled edge of a wheel (the Ganong marker) or with a cotton thread stretched like a bow-string across an arc of springy wire. In the latter case the marker is inked from a pad, and marks 1 to 2 mm. apart are lightly imprinted on the dry surface of the root right down to the apex. Several roots should be thus marked and then carefully planted in damp Coconut fibre, without bending or injury. After twenty-four hours inspect them and note that if growth has been active the marks are no longer equidistant. Those at the extreme apex will not have separated much, if at all, while those at a distance of 3 to 5 mm. from the apex will probably be twice as far apart as at first. From this point, the zone of maximum growth or cell elongation, the distances apart of successive marks progressively diminish until at the base of the root no growth in length is registered.

2. Internodes of young stems, *e.g.*, of seedlings of Kidney Bean, may be similarly marked. In this case the zone of maximum elongation lies towards

the upper end of the internode, while the base does not elongate. Each internode has its own separate growth rate and growth-cycle so that the growth of the whole stem is a composite effect, not a single growth unit as in the root. For this reason, growth studies of whole organs are best made with shoots that have but a single internode or else none, like flower stalks.

3. The growth of young leaves is superficial, not linear, so that a system of grid-markings is required to register growth. A rubber grid stamp is sold under Professor Ganong's name, and is much the most convenient instrument for the purpose. If it is unobtainable, a grid system with 2 mm. squares should be marked with Indian ink on one of the youngest leaves available, using a stretched thread as in paragraph 1. The expansion of the leaf blade is general at first, but later becomes almost entirely marginal, with a zone of growth also at the base. In monocotyledonous leaves like *Narcissus*, growth is linear, and such leaves must be marked with successive intervals instead of squares, paying special attention to the base, where growth is chiefly localized.

C. MEASUREMENT OF GROWTH WITH THE AUXANOMETER

There are many types of instruments designed for measuring and recording growth, varying greatly in complexity and accuracy. As was pointed out in the previous paragraph, it is preferable to use organs which are composed of only one growth-unit for such measurements. The leaves of bulbs, flower stalks, and the hypocotyls of large seedlings like Sunflower are favourite subjects.

Fig. 94 illustrates a simple lever-pattern auxanometer, without any recording arrangement, which will be found very practical for large classes, as it is easily made.

1. One of the most popular auxanometers is Farmer's pattern. This makes a continuous record on a drum covered with smoked paper. The drum is free to move on a vertical axis and has a short arm projecting from the lower edge, carrying a pin which engages the hands of a clock which are also rotating on a vertical axis (see Fig. 95). The clock is provided with four adjustable hands, so that an impulse may be given to the drum at any fixed intervals between fifteen minutes and one hour. When one of the clock hands engages with the arm from the drum the latter is rotated through an arc of about 1 cm. When released by the clock hand the drum is returned to its original position by the pressure of a weight. A recording lever, which may be of any convenient length, is arranged so that its longer arm, carrying a sharp metal point, rests against the side of the drum, while the shorter arm is attached to the tip of the growing plant by a looped silk thread (non-hygroscopic). A magnification ratio of at least 10 : 1 between the arms of the lever is advisable. The drum is covered with smooth-surfaced paper, cut to fit with a small overlap, which is fixed down with paste or gum. The smoking of the drum is carried out by means of a tuft of cotton (or asbestos) wool soaked in Xylol and held in a pair of tongs. When lit this produces

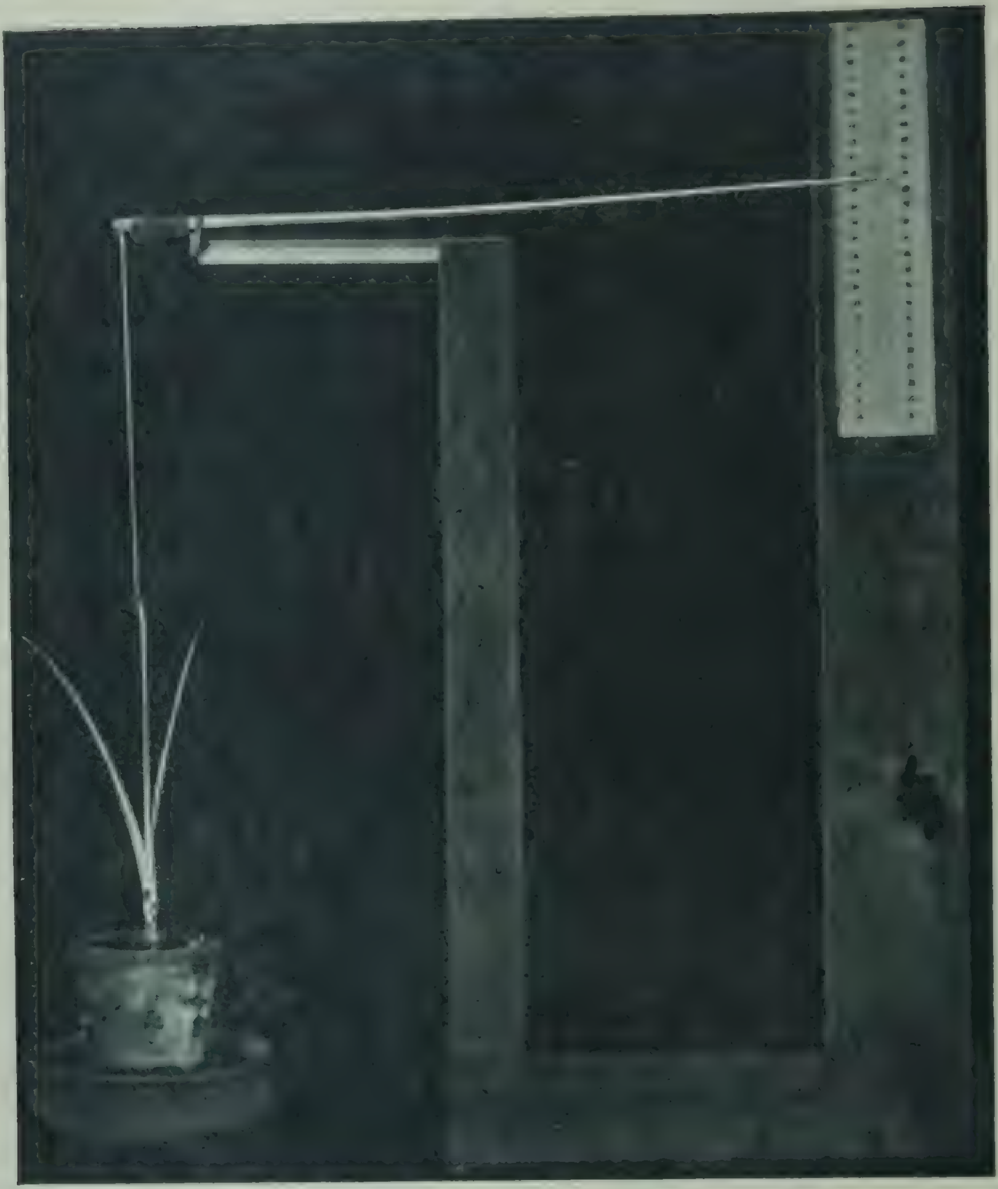

FIG. 94.—A simple lever-pattern auxanometer.

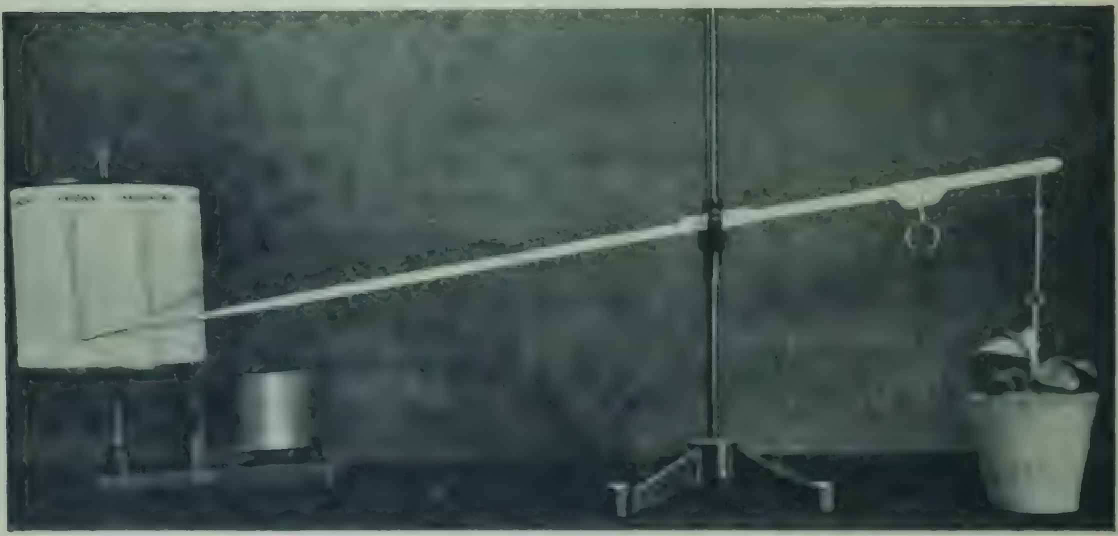

FIG. 95.—Farmer's drum-pattern auxanometer.

volumes of black smoke, and an assistant turns the paper-covered drum in the smoke until it is covered with an even black deposit, which should not be too thick. The operation is a messy one, and is best done out of doors or in a fume cupboard. When finished the soot is wiped off all the parts where it is not wanted and the drum is mounted on its axis on the apparatus. The lever is then adjusted and the time of commencement scratched on the drum at the starting-point. As the plant grows the lever descends and the vertical distance between successive horizontal strokes gives the amount of growth which has taken place in the selected time intervals between the impulses given to the drum by the clock hand. Note that the whole apparatus must stand on a carefully levelled bench, or it will be impossible to keep continuous contact between the lever and drum.

An alternative means of marking is by the use of white paper and an inked barograph pen attached to the lever.

Smoked records may be preserved by cutting out the piece of paper containing the record and floating it, face upwards, on Paraffin wax melted in a shallow dish. The paper is removed, without allowing it to sink, as soon as it is permeated by the wax, which when cold, binds the carbon deposit fairly firmly.

Levers may be improvised from various materials, such as $\frac{3}{16}$ in. aluminium rod, but a split lath of wood is as good as anything. Frictionless bearings may be arranged either by driving a needle through the lath at the fulcrum and supporting the two ends of the needle in small glass tubes, or by bending up a small stirrup of tin, with a hole through it above the lever, swinging on a needle driven into wood.

2. The " Pin hole " Auxanometer of Bedell may be found handy when no specialized apparatus is available. A piece of plywood about 1 ft. by 18 in. has a round hole 2 in. wide cut near one end. This is covered with black paper or blackened tin, through which a single pin hole is pierced. The board is fixed vertically, one end resting on the table, and the potted plant is placed in front of it in such a position that the line of sight through the pin hole allows the tip of the plant to be seen against a bold scale marked on the opposite wall. This scale may be conveniently made from the paper scales sold for covering surveyors' levelling staves. This arrangement provides an optical lever with considerable magnification, by means of which the plant growth may be measured on the scale. If it is difficult to read the markings of the scale through the pin hole, get an assistant to lower a piece of black paper in front of the scale until it appears to touch the tip of the plant. The assistant can then read the level on the scale.

D. ROOT AUXANOMETER

Neilson Jones Apparatus.—The apparatus is illustrated in Fig. 96. It consists essentially of a glass jar fitted with a cork. Through a hole in this cork a glass rod runs freely. A long pin passes through the cork, and on this a seedling is impaled inside the jar. Pour some water into the jar and adjust the height of the seedling by means of the pin until its root-tip just touches the surface of the water. When the glass rod is raised the level of the water falls, and vice versa. Raise the rod and then lower it again until the water just reaches the root-tip, then the position of the glass rod, in relation to a bent-pin pointer fixed in the cork, is marked on it. The apparatus is left for five to ten minutes and a fresh adjustment of the level is made. A difference of 2 to 3 cm. in the position of the glass rod may be found if the root is growing actively. The moment of contact between water surface and root-tip is so well marked that readings of the glass rod should be obtained to the nearest millimetre.

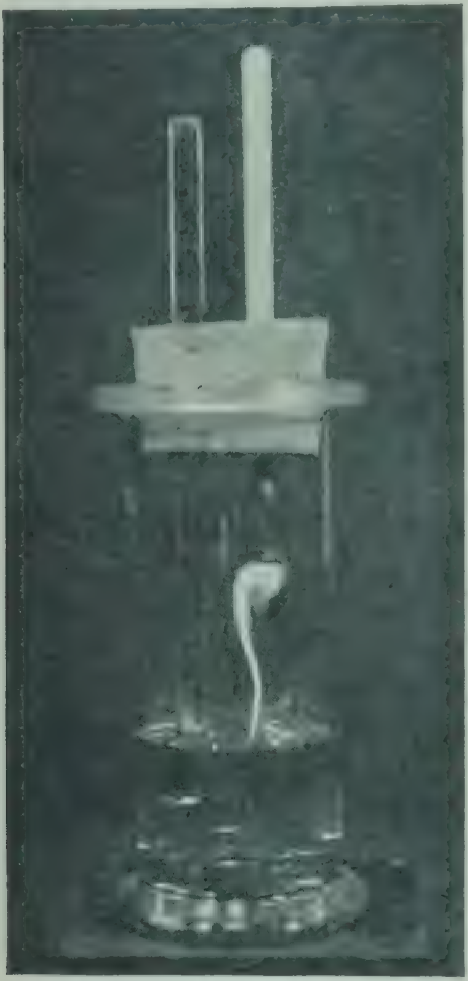

FIG. 96.—The Neilson Jones auxanometer for roots.

The magnification involved depends on the relative cross-sectional areas of the jar and the glass rod. The actual elongation of the root will be given by the difference between the two readings on the glass rod, multiplied by $\frac{s}{S}$, where s = the cross-sectional area of the glass rod and S = the area of the water surface. For example, with a glass rod of 5 mm. in diameter and a jar 5 cm. in diameter, the magnification is 100 times. By means of curved callipers it is easy to measure the internal diameter of the jar at the level of the water surface. Errors due to alteration of the water level by evaporation during long experiments may be avoided by fitting a glass rod, drawn out to a fine point, through the cork. The point is placed at approximately the first level of the root-tip, and each time a reading is made the position of the glass rod which just causes contact of the water with the glass point is also recorded. This provides a constant zero to which the root growth is referred, and alterations of the water level are rendered unimportant.

E. EFFECT OF TEMPERATURE ON GROWTH

The temperature coefficient is the name for the factor by which the velocity of any process is multiplied, for an increase of temperature of 10° C.

It is denoted by the symbol Q_{10}. Organic growth has a coefficient in the region of 2·0 to 3·0, but only over a very limited range of temperature. It can be determined by direct measurement of a growing root if some means of varying temperature is available. A low-temperature oven with a variable setting is the best means, but if that is not available the experiment must be conducted in several different places chosen for their different temperatures, *e.g.*, out of doors, in a corridor, in a greenhouse or in a heated room, etc., the temperature being observed in each case.

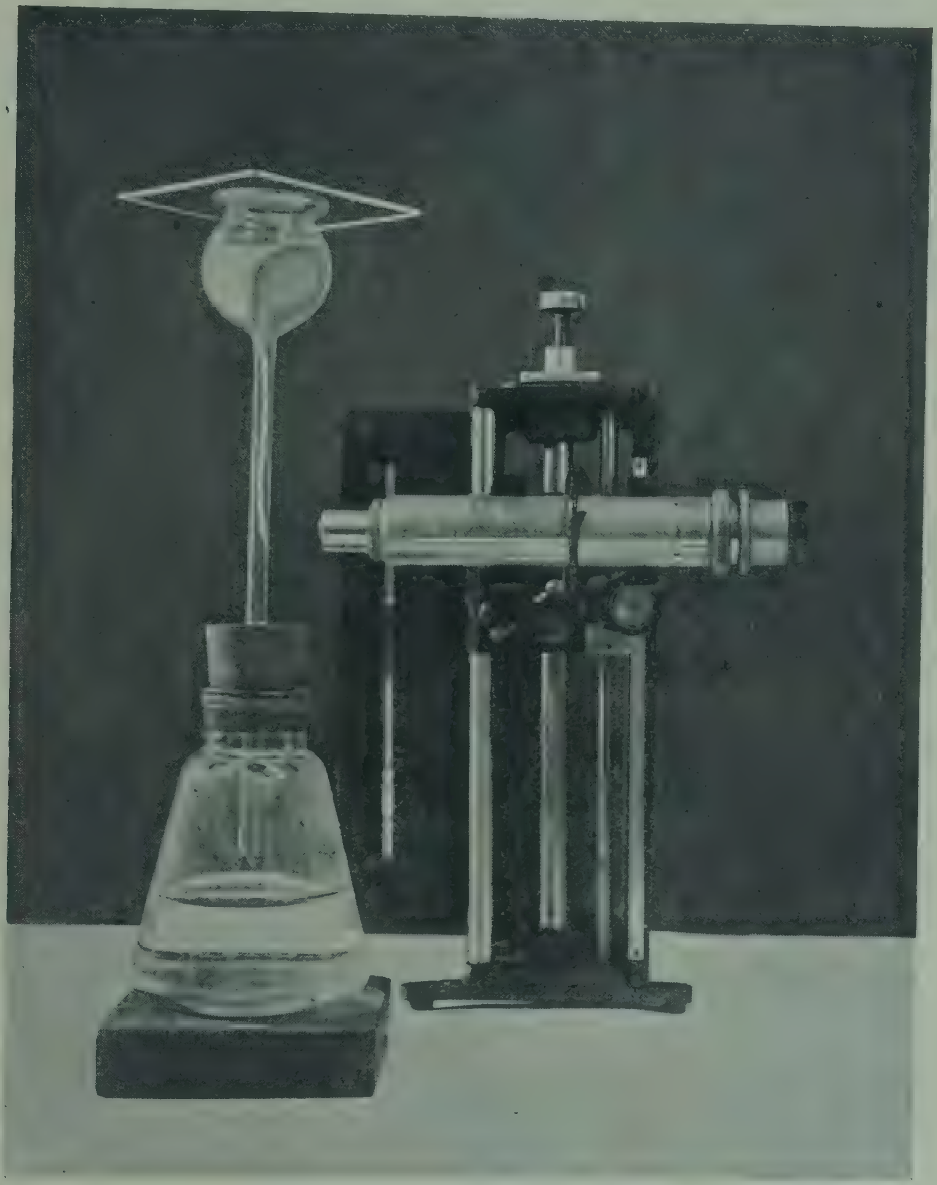

FIG. 97.—Measurement of root growth by a measuring microscope.

Fit a wide-mouthed bottle, of about ½ litre capacity, with a cork bored to admit the stem of a thistle funnel. Half-fill the bottle with water. Place in the funnel a Bean which has been previously sprouted in Coconut fibre till the primary root is about 1 in. long. The root is carefully introduced into the top of the funnel stem, and the seed is then covered with wet cotton wool (see Fig. 97). It is also advisable to cover the funnel with a small square of cellulose film held with a rubber band, so that evaporation is prevented.

The growing root should be surrounded with black paper to keep it dark except when observations are being made.

The observations are simply direct measurements of the increase in length of the root over a given time at a given temperature. If a horizontal measuring microscope is not available, fix a millimetre scale behind the stem of the funnel and read the lengths to estimated tenths of a millimetre by means of a pocket lens focused through a pin hole to avoid parallax.

Intervals of two to three hours will usually be sufficient for good readings, except at very low temperatures. After readings have been made at any given temperature, the cover should be removed to renew the air supply, and fresh cotton wool placed over the seed.

The temperature intervals chosen need not be exactly 10° C., since the coefficient can be calculated to this interval by means of the formula :—

$$Q_{10} = \sqrt[T]{\left(\frac{V_2}{V_1}\right)} \times 10$$

T = Observed temperature difference.
V_2 = Rate of growth at higher temperature.
V_1 = Rate of growth at lower temperature.

Students are warned that the function is not linear, but, as the equation shows, exponential. In other words, $Q_{10} = Q_1^{10}$, and it is, moreover, not a constant for different temperature ranges, but must be separately observed and calculated in each case.

F. CIRCUMNUTATION

As circumnutation is an automatic growth movement it is best treated as a phenomenon of growth. It consists of a rotatory movement of the growing apex, conditioned by the fact that the rate of growth in the young internodes of the stem is not equal on all radii, and that the sector of maximum growth rate moves round the circumference of the stem, so that the rate of growth on any one radius changes rhythmically. Why this occurs is not known, but the effect is to cause a rotation of the apex, either clockwise or anti-clockwise.

To observe circumnutation, a light pointer, made of a thread of drawn-out glass, should be attached to the apex with a spot of soft wax (not sealing wax) so that it stands in line with the tip of the stem. Darwin and Acton recommend that a minute triangle of paper should be slipped on to the pointer and pushed down to its base, while the tip of the pointer is provided with a tiny bead of sealing wax to make it visible against the paper. Above the plant is fixed a sheet of glass about 1 ft. square, held in place by a clamp (see Fig. 98). Through the glass the pointer is sighted, getting the terminal bead in the centre of the paper triangle, and an ink spot made on the glass at the point at which it is aiming at the moment. Add a note of the time at the side of each mark. Repeat the observation at intervals, numbering the marks in sequence so that they may be joined together by a continuous line at the end of the experiment. As twining plants usually show larger and more rapid movement than others, it is well to choose such a subject as a young plant of Kidney Bean or Scarlet Runner, which complete one rotation in about two hours.

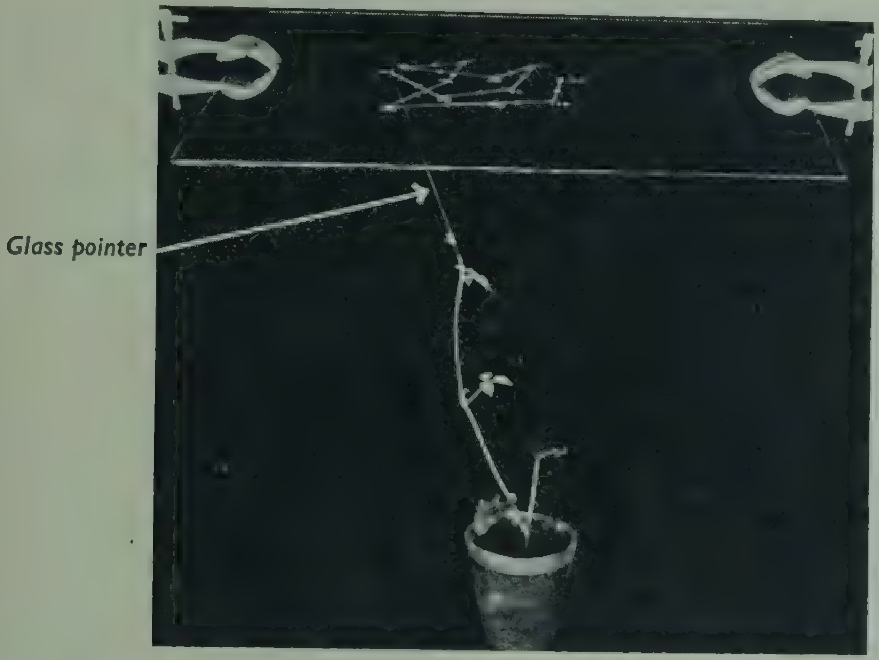

FIG. 98.—Apparatus to map the circumnutation of a shoot.

It is essential to avoid any phototropic curvature during the experiment. The plant should therefore be surrounded by a rolled cylinder of black paper as high as the plant and light directed on it from above by means of a mirror.

G. GROWTH PRESSURE

The elongation of the cells in a growing stem, due to turgor pressure, is a powerful force ; indeed, the ability of growing plants to split rocks and raise astonishingly great weights is proverbial. It may be displayed by even so delicate an object as the slender stem of a Pea seedling.

Plant a young Pea seedling in a 3 in. pot, using sandy soil, pressed down and made firm round the plant, and then watered well. Cut a circle of cardboard the same size as the top of the soil and punch a hole at the centre. Slip this over the plant so that it lies on the soil. On this card stand a piece of glass tubing about 6 in. long and 1 in. wide, which should be clamped firmly, with one end resting on the card and enclosing the plant stem. A specimen tube with a flat base should now be selected, which will slip easily inside the wide tube. Lower it until it rests on the plumule of the seedling. Into the inner tube drop a few lead shot or a little Mercury and leave for some hours in a warm place in a good light. Then add a little more weight to the inner tube (see Fig. 99). Repeat this at intervals of a few hours, so long as the plumule continues to grow and lift the weight. Water at intervals. If the weight added is known and a cross section of the stem is measured for area, the pressure developed may be calculated in pounds per square inch. Pressures of up to 50 lb. per sq. in. have sometimes been recorded. The example figured developed only 15 lb., probably because insufficient time was given for the seedling to establish itself properly in the pot.

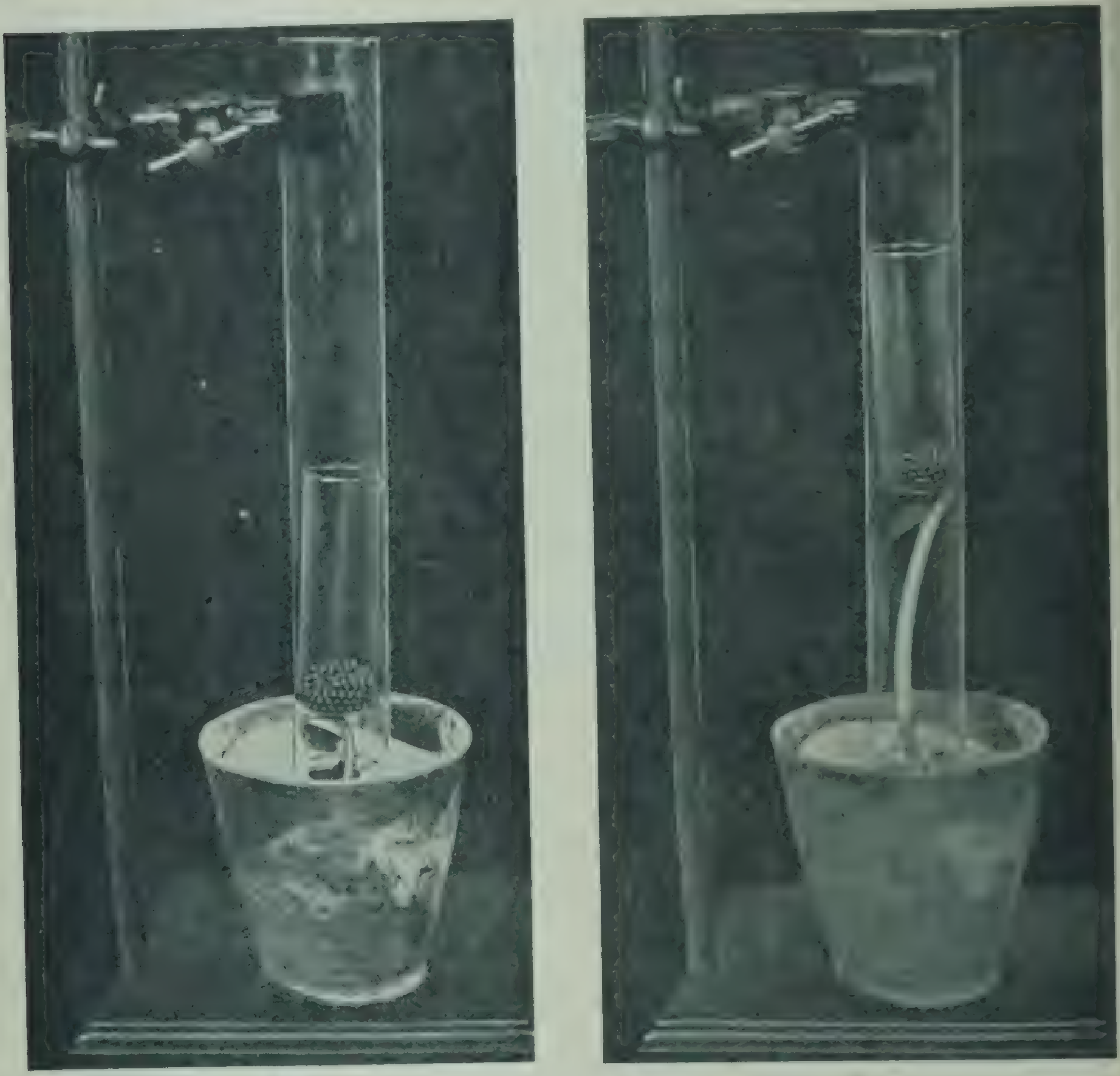

FIG. 99.—Apparatus to demonstrate growth pressure.

H. EFFECT OF GROWTH SUBSTANCES

The development of our knowledge of the effects of growth hormones in the plant and their control of tropic movements has had some interesting practical applications in the use of synthetic substances of analogous chemical constitution for the promotion of growth in cultivated plants. This is particularly well shown in the rooting of cuttings.

Those interested will find full details in three papers by M. A. H. Tincker in the *Journal of the Royal Horticultural Society*, vol. 61, page 510; vol. 63, page 210; and vol. 64, page 554.

1. Test cuttings of various available shrubs or trees, with and without treatment with growth-promoting substances. The most easily available of these are β-Indolylacetic acid (Hetero-auxin) and Indolyl butyric acid. The latter is better. There are also several proprietary preparations containing one or other of the active substances which can be obtained from seedsmen.

A solution of the acid (or of its Sodium salt) is made up, of 1/30,000 strength, and the cuttings are placed with their ends in this for twenty-four to forty-eight hours. They are then placed in wet sand in a frame or cold greenhouse and kept well shaded and watered when necessary. The sand should be frequently changed. Controls are planted in sand directly. Results show in from three to six weeks, according to the species used.

Good plants to try are *Rosa*, *Buddleia*, *Ceanothus*, *Viburnum*, *Myrtus*, *Fuchsia*, *Forsythia* or *Philadelphus*. The months from May to September are the most favourable. A variety of plants should be tried, as some species are very refractory and difficult to root. Others, such as Willows, are so easy that they do not provide an adequate test.

The above concentration is that most generally suitable, but there is a good deal of variation in the concentration required by different plants. The necessary concentrations lie between 1/10,000 and 1/100,000, and the most difficult plants require the stronger doses.

2. *Polarity*.—Willow cuttings may be used to illustrate polarity. Take two cuttings of any Willow, from the younger branches. Trim them to about 6 in. long, remove the leaves and stand the cuttings in a glass jar with about 1 in. of water in it. Place one cutting the right way up and the other upside down. In a week or two adventitious roots will appear and buds may begin to open. Note that in both cuttings the roots come from the basal end, irrespective of its position, and the new leafy shoot from the apical end.

Set another Willow cutting, with about 1 in. of bark removed in a ring near one end. When this sprouts it will be found that each part, above and below the ring, shows independent polarity, roots coming from the basal end of each part and shoots from the apical end. This polarity is supposed to be controlled by the movement of foodstuffs.

Section 8. Response

A. EXPERIMENTS ON GEOTROPISM

A supply of freshly germinated Beans or Peas is required.

1. Take a glass jar and line it with white blotting paper, leaving a narrow slit uncovered to see through. Cut a piece of thin wood or cork sheet to fit standing up inside the jar. Place an inch or so of water in the bottom of the jar, cover the top and leave it for a short while to become thoroughly moist.

Now mark three seedling roots as described on page 360. Fix the seedlings on the wood, with pins through the cotyledons. Fix one with the root pointing downwards as a control, one with the root horizontal and the third with the root also horizontal but with 2 mm. of the root-tip removed with a sharp razor. After a day it will be seen that all three roots have grown. The first should be normal, the second will have curved downwards and it should be noted that the curvature is principally at the region of maximum growth a few millimetres behind the apex. The third will also show growth, but in this case no curvature results, as the perceptive region at the tip has been removed. This result is not due simply to the shock of amputation. If the root is placed horizontally and left thus for some minutes *before* the tip is cut off, a curvature afterwards results, as the perceptive region was able to transmit the stimulus before its removal. Actually perception is not entirely confined to the apex. The growing zone is also perceptive, but much less so than the tip.

2. Make up a 15 per cent. solution of Gelatine and pour into a glass jar or crystallizing dish with straight sides, to make a layer about 2 cm. deep. When it has set solid, place on the surface one or two Pea seedlings, which have straight roots from ½ to 1 in. in length. Place them so that the root lies straight and flat on the Gelatine surface. If necessary, pin them in position with a needle through the cotyledons and into the Gelatine, or, alternatively, to a strip of cork sheet about 1 in. broad bent round inside the dish. Cover with a glass plate. After a day or so the root-tips will be seen to have penetrated into the Gelatine for some distance, showing that the downward movement of roots is a definite growth phenomenon involving work and is not simply due to " drooping " under their own weight.

3. Any quickly growing shoot such as those of *Vinca* or *Polygonum*, or alternatively a young Sunflower seedling, should have the stem marked in 10 mm. intervals. It is then laid horizontally with the cut end immersed in water through a split cork in a bottle, or else stuck into wet sand. Fix a sheet of glass upright beside the plant and mark at intervals on the glass the position of the shoot as it bends upwards. Note that it does not attain the true vertical at once, but over-swings it and then reverses, there being normally several oscillations about the vertical line before it comes to rest.

Measure the distance between the marks at the conclusion of the oscillation and note which part is the seat of response, *i.e.*, that part which has grown most during the experiment.

B. THE KLINOSTAT

This is an apparatus for the investigation of geotropism. It is based on the principle that the only way of neutralizing the influence of gravity is to keep the plant in continual rotation on a horizontal axis, so that gravity affects all sides equally.

Klinostats vary very much in complexity and in cost. The simplest of all may be improvised by soldering an L-shaped piece of thin iron rod to the minute hand of a cheap clock. The horizontal part of the rod may be supported in two holders drilled in the side of a box, and will, of course, rotate once per hour. The older klinostats were all clockwork driven, but the more modern types use an electric motor with worm gears. Some of these types are not at all expensive and are very satisfactory.

1. Fix a small pot containing a young growing Bean seedling on the klinostat and arrange it to rotate about once in twenty minutes. Place beside it on the table another similar pot and seedling, lying horizontal (Fig. 100).

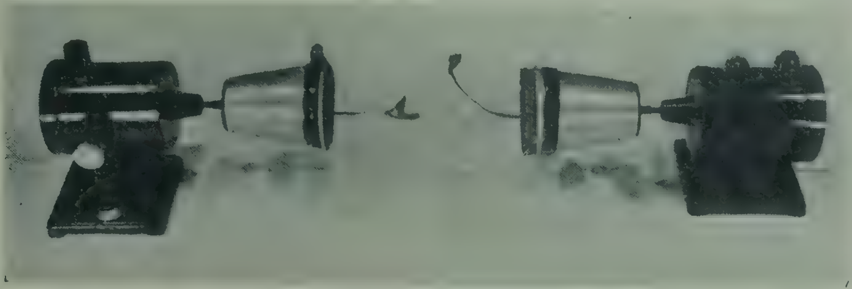

FIG. 100.—Two klinostats (Small's). That on the left is rotating while that on the right is stationary.

Leave for a day. The fixed plant will have turned apogeotropically, but the plant on the klinostat is still horizontal. If you measure carefully the length of the two plants it will be found that the continuous geotropic stimulus of the revolving plant has caused it to grow more than the stationary one. If the clock is now stopped for a few hours the plant on the klinostat will then respond normally and acquire an upward curvature. This should be done in the dark to avoid the complication of phototropic curvature. When the experimental plant has produced a definite curvature start the clock once more. It will be found that after a day the curvature is much diminished or has quite disappeared. This is due to compensating growth and the phenomenon has been called **autotropism** or **rectipetality.**

2. The klinostat also serves for similar experiments on phototropism, if it can be arranged to rotate with its axis vertical instead of horizontal.

C. GEOTROPISM IN RELATION TO RESPIRATION

As geotropic movements, like other tropic movements, are based on differences in the growth rate of different parts of the organism, it follows that energy is required for their occurrence—energy which can only be supplied by respiration. Thus absence of Oxygen inhibits tropic movements.

Grass haulms are useful material for this experiment. The swollen nodes of the stalk are capable of meristematic growth and, when the stalk is laid horizontally, the lower side of the node grows more than the upper side so that the stalk is turned upwards. This provides for recovery from accidents of weather or traffic which so often lay flat the delicate stems of grasses.

Take a large test-tube or specimen tube about 1 in. by 6 in. and fit it with a cork, through which two bent glass tubes are passed, one going to the bottom of the tube, the other stopping short at the cork. Cut a rectangle of plywood or cork sheet to fit inside the tube and to it pin a couple of strong grass haulms cut with a node about the middle of each. Two are used so that one acts as a control on the other. Place a wad of cotton wool over the lower extremities of the stalks, then slip the sheet into the tube, insert the cork and lay the tube horizontal. Attach the long leading tube to a supply of Hydrogen, *e.g.*, a Kipp's apparatus, and pass Hydrogen into the tube for some minutes. Cut off the supply and attach a vacuum pump to the short tube. Exhaust the tube, cut off the pump and again admit Hydrogen. Repeat this two or three times until all air has been removed from the tube, which may be determined by collecting a tubeful of the gas issuing out, which should burn with a quiet flame when all air has been displaced.

Such a preparation will show no geotropic response on the part of the grass stalks. If, however, they are taken out and laid on the table the response will soon be evident, showing that they have not been injured by the treatment.

D. PHOTOTROPIC RESPONSE

One of the most sensitive objects to light stimulation is the coleoptile of the grass seedling, especially that of the Oat, which has been used for most of the research on phototropism in recent years. Most grass seedlings will do, but Oat or Italian Millet (*Setaria italica*) are particularly useful.

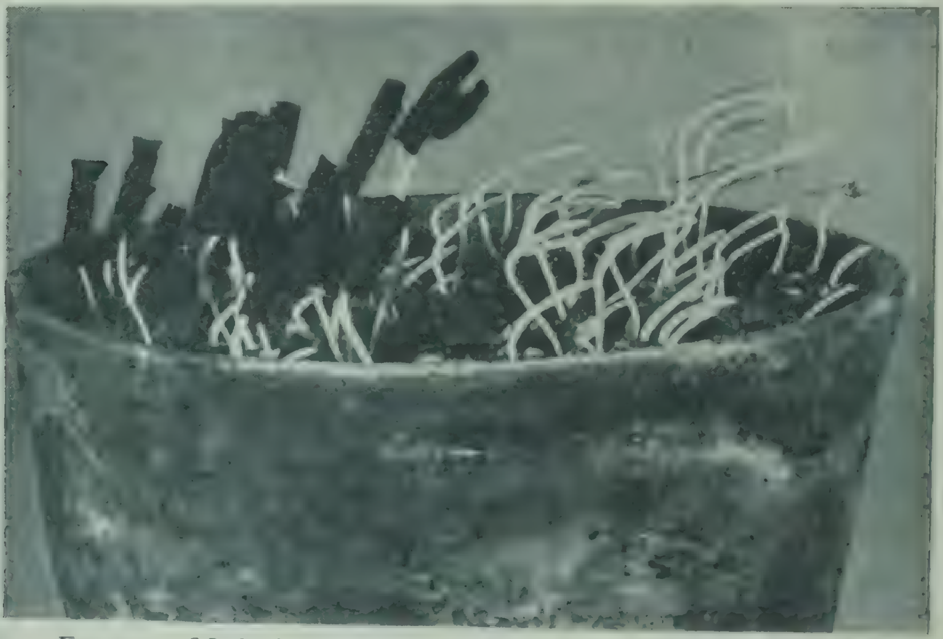

FIG. 101.—Method of demonstrating the action of light on seedlings.

Sow some Oat seed thinly in a 5 in. pot and place it to germinate in the dark. Watch it carefully and use it when the coleoptiles are about ½ in. long. If they have opened and the first leaf has appeared they can no longer be used. Two or three days at the most will be needed to reach the right stage.

With tin foil or black paper and the end of a match stick, cut down to about 1 mm. thickness, as a mould, twist a number of little caps which are to be fixed over the tips of a number of seedlings. Wrap a similar covering round the bases of another group of seedlings, leaving the apices uncovered. Lastly, cover the apices of a third lot with cellulose film.

Expose the pot to one-sided light on a window-ledge or in a box with a hole cut in one side, until next day. It will be seen (Fig. 101) that the seedlings with the dark caps have remained upright. Those covered at the base have responded by turning towards the light, like the untreated seedlings. So have the seedlings capped with cellulose film. This shows that perception is localized in the apex, but that the response which produces movement takes place at the base. The effect of capping the seedlings is principally to cut off light from the receptive zone, and the seedlings capped by the cellulose film show that it is the cutting off of light, not of air or of Carbon dioxide, which makes the difference.

E. PHOTOTROPISM WITH LIGHT OF DIFFERENT COLOURS AND INTENSITIES

1. Such experiments are best carried out in wooden boxes which have a window about 1½ in. square cut in one end and covered by a light screen. The screens may be pieces of coloured glass, which should be tested with a pocket spectroscope to ascertain their light-transmitting qualities. An improvement on this is to use either Wratten Screens (Messrs Kodak Ltd.) or Ilford Screens (Messrs Ilford Ltd.), choosing a red, a green and a blue of, as nearly as possible, equal light-transmitting power. Suitable screens are Wratten : Nos. 25, Red ; 59, Green ; and 49, Blue. If a pot of seedlings is exposed behind each of these colours it will be found that the blue is the most effective, green less so, and that red is practically inoperative.

2. A similar type of experiment will reveal the existence of a minimum threshold intensity for stimulation. For this purpose an electric lamp of low wattage, about 15 watts, is used, with some kind of neutral-coloured screen, such as a photographic plate, developed in a weak yellow light, so as to produce partial darkening. Two or more of these may be combined if necessary. Place the screened lamp at one end of a table in a dark room and place several saucers, in which grass seeds are germinating on wet cotton wool, at intervals along the table. A position will be found beyond which the light intensity is too low to induce stimulation. To get a rough idea of the light intensity at this point expose a piece of gas-light printing paper in a frame at the same distance from the lamp for one minute and develop it for a fixed time, say, two minutes. If this is repeated when different seedlings are used, and the amount of darkening of the papers compared, some idea of the relative sensitiveness of the species may be obtained. Remember that the intensity of light varies as the square of the distance from the lamp.

3. The plagiotropism of leaves, which causes them to set themselves with their upper surfaces normal to the incident light, is a familiar observation to anyone who has seen a Geranium plant growing on the ledge of a small window. The experiment is easily repeated, and it will be observed that the stem is positively phototropic and points directly towards the window, but that the leaves place their surfaces more or less parallel to the window, *i.e.*, transversely to the direction of the light.

F. NYCTINASTISM OF LEAVES

One of the best plants to show nyctinastism is *Oxalis acetosella*, the Wood Sorrel ; commoner plants, less striking in their responses, are *Lotus corniculatus* and *Trifolium repens*. Nastisms differ from tropisms in the absence of any definite relation between the response and the direction, if any, of the stimulus. Experiments may be made either with potted plants or with turves containing the plants, dug up and placed in dishes of water. That light and not temperature is the factor concerned may be shown by covering the plant with a dark shade, when it will assume the sleep position at any time of day. If the plants are kept continuously in the dark for several days the leaves will eventually move back spontaneously into the daylight position or nearly so.

If *Oxalis* is placed in bright sunlight the leaves will also assume the sleep position ; that is to say, there is an upper limit to the light intensity which induces the day position, beyond which the movement is reversed, as in phototropism.

If the plants can be shaded by covers made of coloured glass, or of coloured cellulose film on a framework, it will be found that blue acts like daylight and red like darkness (Fig. 102). Nyctinastic movements are also shown by many flowers, some closing at night, such as Tulip, Crocus and Daisy, while others open at night, *e.g.*, *Nicotiana* and Night-Scented Stock. In some cases light, in other cases temperature changes, are the motive factor. If you have a garden you can easily experiment for yourself by darkening flowers in the daytime or warming them (if in pots) indoors at night, and observing which treatment causes a change of position.

G. HYDROTROPISM OF ROOTS

The positive curvature of roots towards a supply of water is sufficiently strong to overcome even geotropism.

1. Take a shallow box about 6 in. square, knock out the bottom and replace it with either a small-mesh wire netting or coarse perforated metal sheet. Place about 1 in. of damp Coconut fibre in the sieve thus formed and on it sow a number of Mustard seeds. Cover the seeds with another shallow layer of fibre. Fix the box in a clamp at an angle of 45° in such a position that a tap may drip gently on to the fibre to keep it wet. The seeds will germinate in a few days and the rootlets will appear through

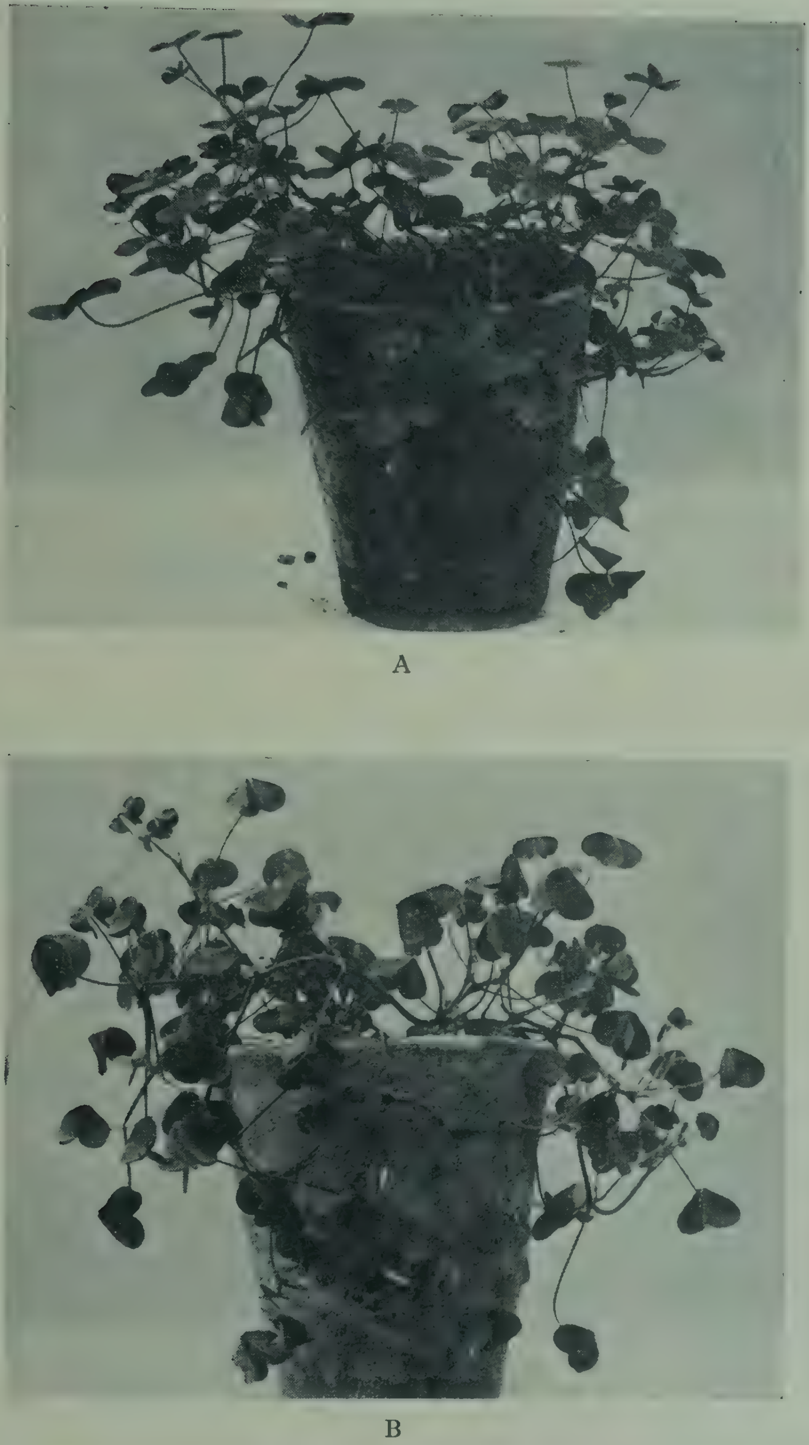

FIG. 102.—Nyctinastic movement in the leaves of *Oxalis corniculata*.
A in daylight ; B in red light.

[*Oxalis corniculata* should not be confused with *Lotus corniculata* (p. 374, Sect. F). The two plants are of course quite distinct.]

the meshes in the netting, but instead of growing straight downwards they will turn aside and apply themselves closely to the wet under-surface of the sieve. Some may even turn upwards and penetrate into the fibre again (see Fig. 103).

2. Fill a box with sandy soil, well damped, and in the centre plunge a small empty flower pot with a cork in the bottom hole. Round the pot, at about 3 in. distance, sow a ring of Beans. Fill the pot with water and keep it filled. When the Beans germinate it will be found that the roots grow towards the pot, from which the water slowly diffuses, and wrap themselves round it, often forming a closely woven network encasing the outer surface of the pot.

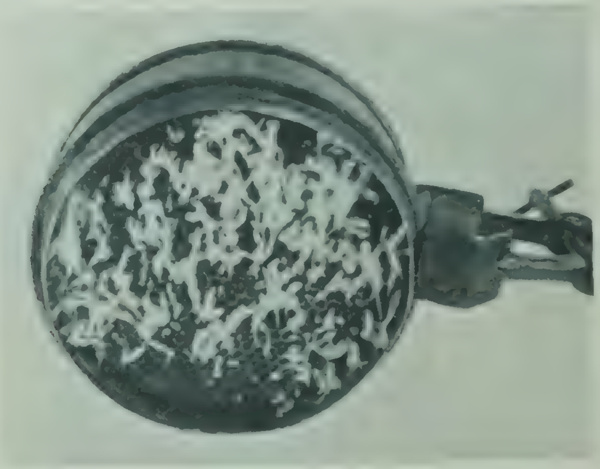

FIG. 103.—Demonstration of hydrotropism in roots.

H. CHEMOTROPISM AND CHEMOTAXIS

Chemical attraction or repulsion plays a decisive role in at least two of the major biological processes, namely, fertilization and parasitism. The following experiments illustrate both principles :—

1. The chemotropic response of pollen tubes towards the stigmas may be demonstrated by germinating suitable pollen in a moist chamber in the presence of stigmatic tissue (see also Part V, Section 3, p. 254).

Take a slide with a ring of metal or glass cemented on it, or else cut a piece of thick blotting paper to the size of a slide and cut out a ½ in. hole in the middle of it. Clean a ¾ in. circular cover-glass and place a small drop of Sucrose solution at the centre. Into this stir some pollen, a small piece of stigma and one or two ovules, all from the same flower or the same species. Invert this over the ring or over the hole in the blotting paper and fasten it down by brushing a little melted vaseline round the edge. Where the paper has been used a few drops of water should be applied to moisten it. Place the slide in a Petri dish or in a covered saucer and keep in darkness for a few hours. As the pollen germinates it will be seen that the ends of the tubes direct themselves towards the stigmatic tissue or the ovules.

Good material for the purpose and the appropriate sugar concentrations are given below :—

Species.	Sucrose Per Cent.
Plantago media . . .	1
Tulipa spp. . . .	3
Narcissus spp. . . .	7
Scilla non-scripta . .	10
Lathyrus odorata . .	15
Echeveria retusa . . .	15
Tropaeolum majus . .	15

Some of the above species (*Lathyrus* and *Echeveria*) begin germination within a few minutes.

2. The chemotaxis of Fern antherozoids may be shown by using weak Sodium malate solutions or with Bacteria by using dilute beef extract solutions.

Glass tubing is drawn out to a fine capillary about 0·1 mm. diameter and broken into centimetre lengths. These are sealed at one end and dropped into a small bottle containing the test fluid. For antherozoids this should be 0·01 per cent. Sodium malate, while for Bacteria any weak broth will do. Attach the bottle to an air pump and exhaust. This will cause the tubes to fill with the fluid when the pump is disconnected.

Young Fern prothalli are easily raised on potted sandy soil, kept moist and closely covered with a piece of glass. Spores are usually present spontaneously, but they may be sown from a piece of mature frond laid on the soil surface, sori downwards, for a few hours. Wash a prothallus carefully with a brush and mount it in a drop of tap water. The antherozoids will be more readily shed if the prothallus is allowed to dry slightly before mounting. Introduce one of the capillaries under the edge of the cover-glass so that the Sodium malate can diffuse into the water. Pfeffer records having caught in this way upwards of a hundred antherozoids in one tube.

A bacterial suspension is most easily obtained by allowing a few Peas to decay in tap water. The diffusion from a broth tube, introduced under a cover-glass, will soon bring the Bacteria swarming around the entrance.

3. Chemotropic stimulation of the hair tentacles of the leaf of *Drosera* is normally brought about by the diffusion of nitrogenous compounds from the prey, but it can also be brought about by nitrogenous compounds directly applied. Proteins, such as coagulated egg-white, will provide a strong stimulus if applied in very small pieces to the ends of the tentacles.

Simple compounds of nitrogen, however, are also effective. Darwin showed that the " inconceivably minute quantity " of 0·000003 mg. of Ammonium phosphate was enough to cause the inflection of a tentacle. This sensitiveness may be tested by applying small drops of very dilute solutions of Ammonium phosphate or Ammonium nitrate to the tips of the lateral tentacles. The central tentacles are much less sensitive than the lateral ones, and 0·068 mg. was needed, in Darwin's experiments, to excite bending of the exterior tentacles when applied to the disc of the leaf. Chemically inert materials such as cork, sawdust, sand, etc., when applied to tentacles do not excite curvature, but the mechanical irritation may cause increased secretion from the glands.

J. CONTACT SENSITIVENESS (THIGMOTROPISM)

Tendrils of climbing plants all show this character in greater or less degree. Those of the Passion-flower are among the best, but those of the White Bryony (*Bryonia dioica*) are quite useful.

The tendrils are only stimulated by a rough contact. Dip a glass rod for a moment into a Gelatine solution and allow it to set. Select a tendril which has already begun to curl at the tip and stroke the concave side with the Gelatine-coated rod. No curvature results. If now the rod be dipped in powdered emery or fine sifted sand and the concave side of the tendril again gently rubbed with the roughened surface, the tip will begin to curl and within a few minutes will have completed a circle.

K. SHOCK SENSITIVENESS

The best subject for these experiments is *Mimosa pudica*, the Sensitive Plant. Seeds can be obtained from all first-class seedsmen, and are not difficult to raise. The plants require a good deal of warmth, but even without a greenhouse they can be kept on a sunny window ledge, at least for the summer, and they provide a great deal of interest. Cover the plant, if possible, with a bell jar when not in use. This prolongs its life.

Each leaflet of the compound leaves has its own minute pulvinus, and a large pulvinus connects the leaf rachis to the stem. All the pulvini are motor organs, and by rapid loss of turgor pressure on one side, when stimulated, they cause the bending movement which has gained the plant its name.

1. Strike a leaf lightly with a stick. The leaflets will fold upwards, but the leaf as a whole turns down (Fig. 104). This is not merely drooping, for if the pot be suspended with the plant downwards the leaf movement becomes an upward one. Touch a pulvinus gently with a needle point. Note that only a stimulus on the lower side is effective.

If the extreme tip of a leaf is pinched with forceps a wave of stimulation can be seen to pass down the leaf, each pair of leaflets closing in turn.

The reaction of *Mimosa* is somewhat dependent on temperature. Thus at 10° C. the response is abolished, while at 15° C. there is usually no response, or only a slight one. The optimum temperature is between 25° and 30° C., and this should be noted when experiments are being carried out.

A mechanical stimulus may be replaced by an electrical one if the electrodes from the secondary of an induction coil are touched to the plant.

The plant takes from ten to twenty minutes to recover itself, and is then ready for restimulation, although it takes a somewhat longer time to regain its maximum sensitiveness. If, however, the stimuli are repeated regularly the plant becomes fatigued and the response becomes less and less. Under continued rapidly repeated stimulation the leaves eventually recover after about fifteen minutes, and thereafter remain insensitive for a considerable time. Sensitiveness is also abolished by keeping the plant in the dark for three or four days.

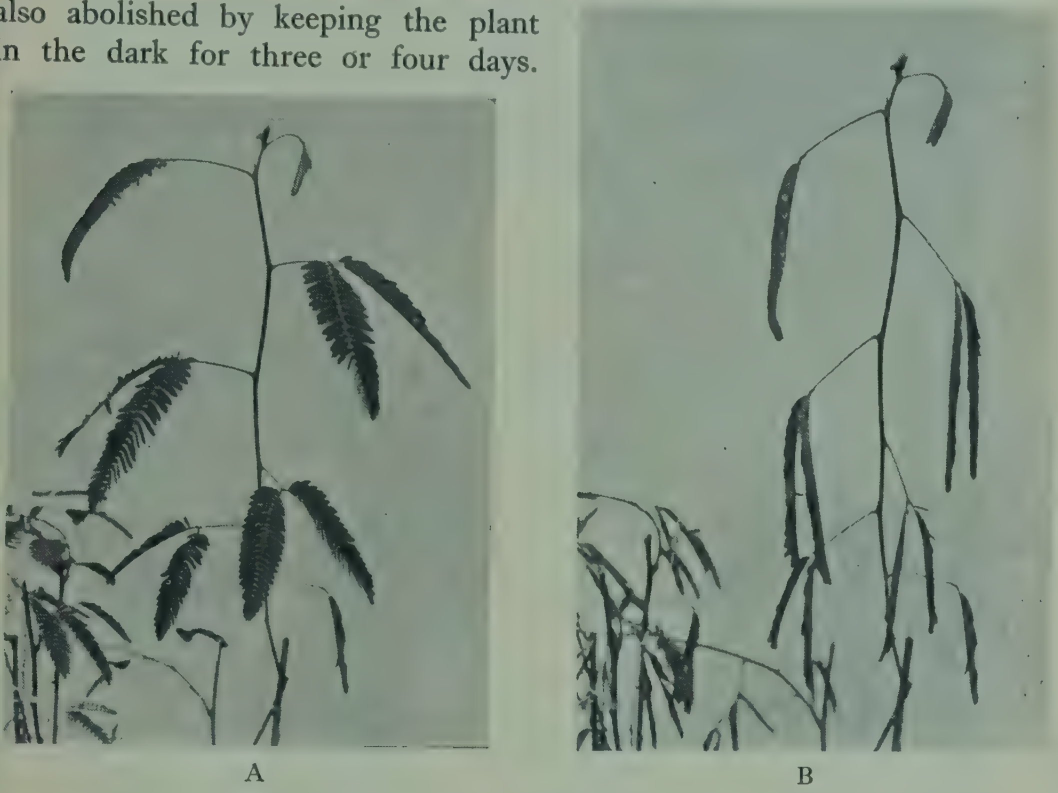

FIG. 104.—Comparison of a plant of *Mimosa pudica* before and after shock.

2. If *Mimosa pudica* is not available, the flowers of one of the garden species of *Berberis* will serve to show shock-sensitiveness, in this case in the stamens. The base of the stamen filament, on its inner side, is sensitive, and if touched with a needle point it shrinks by loss of turgor, so that the whole stamen moves suddenly inwards, bringing the anther against the stigma, in the centre of the flower. Self-pollination does not usually result from this, because the flower is protandrous, but the movement serves to shake pollen on to the head of any insect visitor whose proboscis has reached to, and stimulated, the sensitive part of the filament.

L. HYGROSCOPIC MOVEMENTS

These sometimes affect the whole plant, for example, in the " Rose of Jericho," in which the rosette of branches opens or closes into a ball according to the atmospheric humidity. More usually they are confined to reproductive structures and are often concerned with the dispersal of propagative cells.

Examples are the annulus of the Fern sporangium, the peristome of Mosses, the elaters of Liverworts, the epispore of *Equisetum* and the anther wall of the Angiosperm.

In some cases such movements play an important part in the dispersal of seeds. The hygroscopic awns of *Stipa* and *Erodium* are such cases. The former (*Stipa tenacissima*) is easily grown in gardens, the latter is a British wild plant.

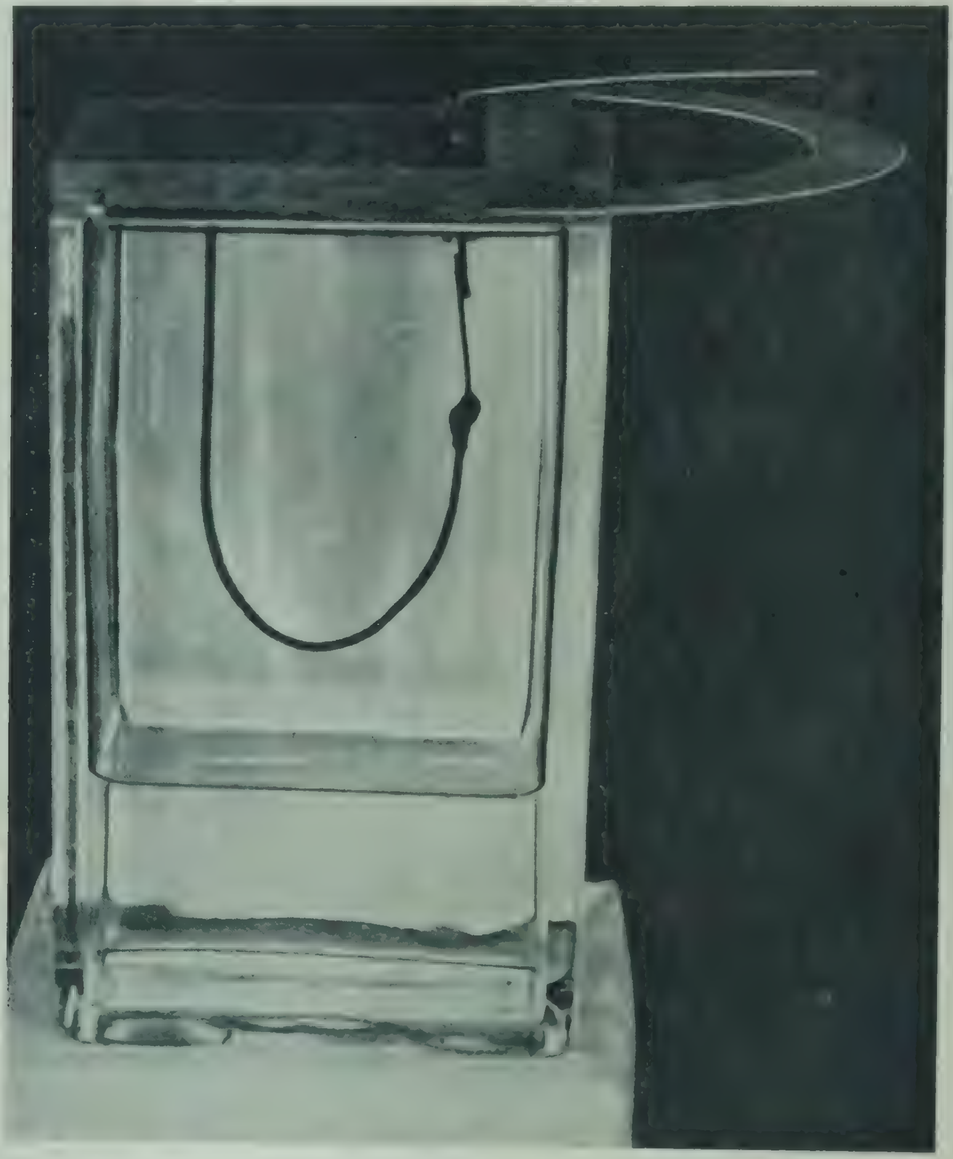

FIG. 105.—Apparatus to demonstrate the hygroscopic movement of the awn of *Stipa tenacissima*. One end of a length of awn (right) is fixed to a stout wire; the other end to the pointer.

1. Examine some of the ripe fruits of *Erodium* (e.g., *E. cicutarium* from sand dunes) in which the awn is spirally wound, with a long curved "tail" standing out at right angles. Place them on a layer of cotton wool in an open dish and leave them exposed to the air, watering at intervals. The movements of the tail soon drive the seed downwards,

the spiral acting as a corkscrew, so that eventually all are buried in the cotton wool.

2. The awn of *Stipa* is larger, with a practically straight tail which is at right angles to the basal portion. The upright basal portion twists and untwists as the humidity of the air changes, so that the tail can be used (over a circular card graduated in degrees) as a hygrometer (Fig. 105). This movement also tends to thrust the spikelets containing the seeds into the ground, being aided by the diverging hairs on the spikelet itself, which act as anchors and prevent the spikelet from being dragged up again.

M. CYCLOSIS

The circulation of protoplasm in cells shows responses to external factors in various ways (see p. 33).

1. Mount a leaf of *Elodea* in tap water and wait till cyclosis has re-started and is in full swing. Time the rotation in a measured cell by observation of a moving plastid and work out the velocity. Now place the slide over a vessel of warm water for about fifteen minutes. Dry the moisture off the slide and examine again, re-calculating the velocity. This is purely a qualitative demonstration of the temperature effect. A quantitative estimate of the Temperature Coefficient (see p. 364) will require some form of hot stage, which enables you to examine the slide while it is being warmed to a definite temperature. There are several types of these on the market. A cheap substitute can be made out of a copper strip about $9\frac{1}{2}$ in. long by 1 in. wide by $\frac{3}{16}$ in. thick. A 4 in. length of this is bent at right angles to hang down at the side of the microscope stage into a beaker of hot water. A hole is bored at the proper point over the opening in the stage of the microscope, and the other end is bent over into a roll to enclose the bulb of a thermometer. This will give an approximate idea of the temperature of an object laid on the heated strip. The slide itself should be covered with a strip of felt with a hole cut in it for observations.

2. That respiration energy is required to maintain the movement may be shown very simply by mounting the leaf in liquid Paraffin. The absence of air soon stops the movement. This is much simpler than using a special slide with leading tubes and passing hydrogen over the leaf, which is the method generally described.

Narcotics also stop the movement. Shake up a few drops of Ether in 100 c.c. of water and mount a leaf in the Ether water.

In both the above experiments movement may be restored by washing the leaf and removing it to a drop of fresh tap water.

PART EIGHT

MODIFICATIONS OF THE NORMAL PLANT FORM

Section 1. Anatomical Modifications

A. LEAVES

1. XEROPHYTES

Erica cinerea (Heather).—Transverse section of leaf. Stain in Aniline chloride and mount in Glycerine.

The leaf margins are **revolute,** that is to say, rolled downwards and inwards toward the midrib, so that two channels, lying one on each side of the midrib, are partially enclosed. The inrolled edges nearly meet the ridge of the midrib below the leaf, so that the channels are almost completely covered. These spaces are densely filled with short **unicellular hairs,** and it is only in these protected spaces that the stomata are to be found.

The upper surface of the leaf appears to be almost flat because two ridges are developed, one on each side, just at the position where the edges bend downwards. These ridges form the two apparent edges of the leaf, as distinguished from its real edges, which are underneath, as described.

Internally, the tissues are little modified from the usual bifacial type. There is a palisade layer and a spongy mesophyll. The **epidermis** is strongly cuticularized and the **cuticle** is markedly ribbed.

The characters suggest that the water loss from such a leaf would be very limited, but it should be understood that such deductions are not always safe and must be submitted, when possible, to the test of experiment. Plants in which the characters suggest, as in this case, the restriction of transpiration are called **xeromorphic.**

Lavandula vera (Lavender).—Transverse section of leaf.

The leaf edges are **revolute,** but not so markedly as in *Erica,* while additional protection is afforded by a covering of **branched hairs** all over the leaf. Each hair has a straight, very much thickened stalk cell, at the top of which is a flat star-shaped head of branch cells. As the stalk cells are of different heights, the branches of neighbouring hairs overlap each other and make a fairly dense canopy over both surfaces of the leaf.

The mesophyll of the leaf is simple, consisting of loose parenchyma throughout, with no distinctive palisade layer, the tissue being merely somewhat more spongy on the lower than on the upper side. In spite of the extra protection afforded by the hairy covering, the **cuticle** is at least as thick as on the glabrous leaf surface of *Erica.*

Hakea pectinata.—Transverse section of the leaf.

Hakea has leaflets not unlike Pine needles in appearance, and their structure is **centric** not bifacial (Fig. 106). There is only one small, central, vascular bundle accompanied by a strong strand of sclerenchyma cells. Surrounding the bundle is a tissue of large parenchyma cells, probably **water-storage tissue.** Some of these cells are filled with prominent brown **tannin masses.** Isolated strands of sclerenchyma occur here and there at the periphery of this parenchymatous zone. The outer zone of the leaf consists of two concentric layers of **palisade cells,** the outer layer with darker contents than the inner. The cells of the inner layer are loosely attached to each other by short connecting processes, leaving a considerable amount of intercellular space. The outer layer is more compact. Across both these layers run radially a number of prominent **sclereids,** or thickened fibre cells, which spread out starwise at both ends and function apparently as mechanical supports for the assimilating layer.

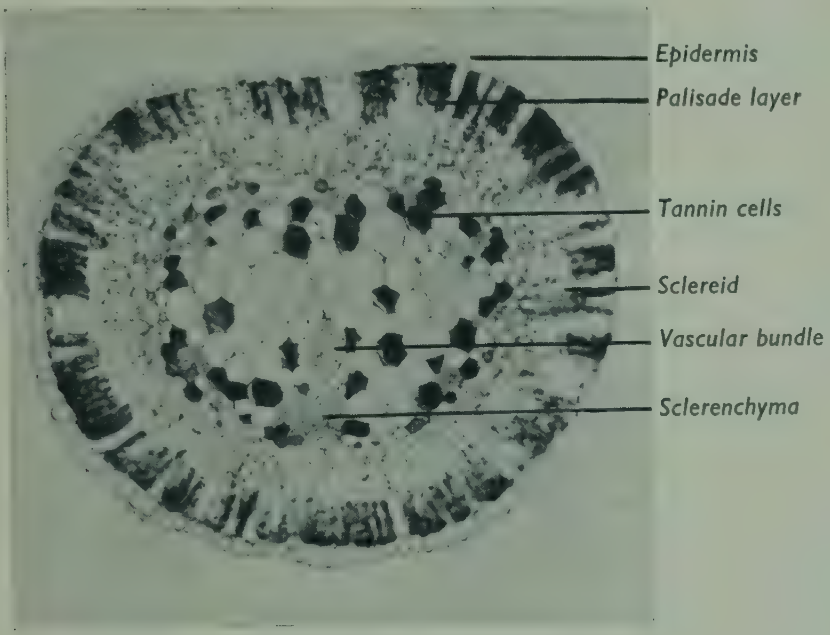

FIG. 106.—*Hakea pectinata.* Transverse section of the leaf.

There is a very strong **epidermis** with thick **cuticle.** The **stomata** are deeply sunken, below the level of the epidermis itself and arched over by the surrounding epidermal cells, so that they are almost enclosed, at the bottom of a conical cavity, through the neck of which is their only connection with the atmosphere. There is a large **assimilation chamber** or space in the mesophyll below each stoma.

A leaf so organized will lose water only very slowly.

Sempervivum tectorum (Houseleek).—Transverse section of the leaf.

A succulent leaf is usually, as here, of very simple construction, water-storage tissue predominating.

The section shows usually one small central and two to four smaller lateral **vascular bundles.** The rest of the **mesophyll** is formed entirely of spherical thin-walled parenchyma, with no differentiation. There are a few **tannin cells** marked by dark glassy-looking contents. The **epidermis** has a cuticle which is heavy, though not unusually so, while the stomata are not in any way sunken or covered up, but are distributed all round the leaf and each has an assimilatory chamber below it.

Ammophila arenaria (Marram Grass).—Transverse section of the leaf.

The plant has very long rhizomes (see Vegetative Propagation, p. 408), and the long sharp-pointed leaves have the property of rolling up like a straw when dry and opening out flat when moist. Many grasses have a similar power of rolling or folding their leaves, though usually in a lesser degree. A section in 50 per cent. Glycerine will be about half unrolled.

The convex side (dorsal side) is really the ab-axial or lower surface of the leaf, as evidenced by the position of the xylem in the vascular bundles. This side is exposed when the leaf is rolled up and presents an unbroken surface of highly thickened **epidermis.** The concave (ad-axial) surface has a number of conspicuous **ridges** with furrows between, and is covered with short stiff **hairs.** Each ridge contains a **vascular bundle** of the monocotyledonous type, surrounded by a single layer of sclerenchyma cells which are excentrically thickened on the inner side of each cell and show fine branching pits. All the ground tissue is of thick-walled parenchyma, except for patches of **chlorenchyma** on the sides of the furrows. The chlorenchyma is all of loose texture without palisade cells, and associated with this tissue the **stomata** are exclusively found. Observe a stoma with the high-power. It has very small and highly thickened guard cells, sunk below the surface of the epidermis on each side.

The basal angle of each furrow is occupied by a group of colourless thin-walled cells rather larger than the other epidermal cells, known as the **hinge cells**. These delicate cells naturally suffer from water loss during drought, and their consequent loss of turgor permits the elastic inrolling of the leaf margins, thus completely enclosing and protecting the furrowed stomatal surface. The turgid expansion of these cells when moist conditions again prevail once more, opens out the rolled leaf and exposes the stomata, which, however, are, even under these conditions, protected by the hairs.

Phormium tenax (New Zealand Flax).—Transverse section of the leaf.

The vertical leaves are stiff and very long, sometimes as much as 6 ft. They contain abundant **sclerenchyma strands** which are sometimes used as a commercial fibre. The leaf is somewhat dorsiventral, unlike many other monocotyledons with vertical leaves, and the ventral side is markedly ribbed as compared with the dorsal. These ribs are caused by the presence of two sets of sclerenchyma strands, one series of large strands, broad enough to reach from side to side of the leaf, which alternate irregularly with a second series of small strands which occur on one or other or on both sides of the leaf separately.

The large strands are associated with the vascular bundles, one of which is embedded in the middle of each of them. The bundles are of the usual bilateral type, with no cambium, but the larger ones have much more xylem than is usual in monocotyledonous bundles, probably owing to the unusual length of the leaf. A few of the smaller sclerenchyma strands also have small bundles at their inner edges.

Each fibrous strand is sheathed in a layer of large rounded cells with thin walls, which separates the strands from the mesophyll proper. This may be a storage layer for the sugars of photosynthesis.

The true mesophyll is divided into blocks by the large strands. Each block consists of two masses of **chlorenchyma,** *i.e.*, parenchyma cells containing abundant chloroplasts, but not in palisade form. One such mass lies towards each face of the leaf, with a single layer of similar cells running alongside the sclerenchyma strands and connecting these two lateral cell masses. The central core of each mesophyll block consists of large thin-walled water-storage cells or **hydrome.**

On the dorsal side there is a strong, continuous, double-layered **hypoderm,** intervening between the strands and the **epidermis,** which is itself very strongly cuticularized. On the ventral side the hypoderm is relatively slight and discontinuous, while the strongly cuticularized epidermis shows rows of very narrow and deeply sunk stomata, each with a minute assimilatory chamber beneath it, confined to the spaces between the sclerenchyma strands.

It is obvious that the leaf is mechanically supported by the strongly developed sclerenchyma, and this is true of many other monocotyledonous leaves. Monocotyledons, as a class, utilize sclerenchyma much more than xylem as a mechanical tissue, a condition associated with the " closed " type of vascular bundle and the consequent absence of secondary thickening.

2. AQUATICS

Nymphaea alba (White Water Lily).—Transverse section of the leaf; stain in Aniline chloride and mount in Glycerine.

The large leaves of the Water Lily float on the water surface and their structure has been considerably altered from that of the normal aerial type of leaf. The **upper epidermis** is slightly cuticularized and contains all the **stomata,** which are numerous and set flush with the cuticle. There is an **hypoderm** of a couple of layers of parenchyma cells and beneath this a broad zone, occupying most of the middle of the leaf, of widely **lacunar tissue.** The lower side of the mesophyll consists of a zone, ten or a dozen cells deep, of relatively small rectangular cells with very thin walls and very small chlorophyll content. The **lower epidermis** has also very thin walls and no cuticle. Grouped around the vascular bundles are most remarkable branched **idioblasts** or " cells of special form." They are lignified cells which send out strong, pointed branches, in all directions, many of them projecting freely into the intercellular spaces, while others resemble pillars, penetrating the lower thin-walled zone of the mesophyll. These idioblasts are so characteristic that the sections can be recognized by them alone. They also occur in the petioles.

3. SUN AND SHADE LEAVES

Fagus sylvatica (Beech).—Transverse sections of leaves; stain in Aniline chloride and mount in Glycerine.

Compare transverse sections of the lamina in leaves chosen from those in full sunlight and those in deep shade respectively.

The Beech tree has very "plastic" foliage. That is to say, it shows marked differences of structure according to the conditions under which it develops, especially the amount of light. The Beech produces a very large number of leaves, and those on lower or inner branches are usually heavily shaded.

The shade leaf is only about two-thirds of the thickness of the sun leaf. In the latter the **epidermal cells** are thick-walled and have a strong cuticle, while in the shade leaf the epidermal walls are delicate and only slightly cuticularized. The **palisade layer** in the sun leaf makes up 30 to 40 per cent. of the total thickness of the lamina. Its cells are six to eight times as long as broad. In the shade leaf the palisade is only about 20 per cent. of the leaf thickness, and its cells are stumpy, about three to four times as long as broad and very loosely arranged. The **spongy tissue** in the shade leaf is reduced to a few more or less isolated strands of cells, and the empty spaces between them amount to something like 75 per cent. of the leaf volume. The **vascular tissue** in the veins is also much reduced, and the smaller veins show very little xylem lignification.

The **stomata** in the shade leaf are fewer and the guard cells somewhat smaller than in the sun leaf, but they are not markedly different in form or in position.

The Horse Chestnut or even the Bracken Fern will afford similar comparisons.

B. STEMS

1. XEROPHYTES

Sarothamnus scoparius (Broom).—Transverse sections of an internode of the green stem; stain in Aniline chloride and mount in Glycerine.

The **wings,** usually five, contain chlorophyllous tissue and perform in part the functions of leaves. The latter are very small and last for only a few weeks each spring, during which time, however, they are physiologically very active. In compensation for the reduction of the leaves the stem has chloroplasts in the cortex, while its total surface is increased by the wings, and therefore its efficiency for photosynthesis is also increased.

Centrally is a large-celled **pith (medulla)** surrounded by a **xylem** ring in which patches of thick-walled sclerenchyma are conspicuous. These provide the extra stiffening which is needed by such long, thin " switch " stems as those of Broom. Outside the xylem there follow, in order (*a*) a narrow **cambium** ; (*b*) **phloem** ; (*c*) groups of **pericycle fibre** cells ; (*d*) **phloeoterma** [1] layer, with some starch grains and a little chlorophyll ; (*e*) **cortex,** five or six cells thick, with abundant chloroplasts in the cells ; (*f*) **epidermis,** with an outer cuticle as thick as the cells themselves and containing stomata.

The wings show one or two median sclerenchyma bundles, while the outer edge of each wing is formed of a bundle of cells so highly thickened that the cell lumen is reduced to an almost invisible central dot. On both flanks of each wing is a **chlorenchyma tissue** containing many chloroplasts. The epidermis outside this tissue shows many **stomata,** with highly thickened guard cells, and each surrounded by a projecting ridge of cuticle.

There is no vascular tissue in the wings, which consist, therefore, morphologically of **extra-stelar** tissue only, namely, cortex and epidermis.

If possible, cut also a transverse section of a leaf.

The mesophyll shows palisade cells towards both surfaces (**bifacial structure**). There are also stomata on both sides. Spongy mesophyll occurs only in the middle layers.

Alternative types : *Spartium junceum* (Spanish Broom) or *Ulex europaeus* (Gorse).

2. AQUATICS

Elodea canadensis.—Transverse section of stem. Mount in Glycerine.

The section shows a great simplification of the histology as compared with land plants. The **epidermis** has no cuticular covering and is practically indistinguishable from the cortex. The **stele** is small and central, with an endodermis which shows a good **Casparian band** on its radial walls. A pericycle is not distinguishable, and the internal tissue of the stele consists of more or less parenchymatous **phloem** elements. In the centre is an intercellular cavity, marking the place at which a single vessel is formed at the growing point. This, however, is resorbed in older parts of the stem, leaving only the cavity, so that no xylem at all may be present.

Myriophyllum verticillatum (Water Milfoil).—Transverse section of stem.

There is a well-marked **epidermis** of small cells with some **cuticle,** then comes a parenchymatous **outer cortex,** followed by a **middle cortex** with large intercellular spaces separated by thin cell membranes arranged radially like the spokes of a wheel, and lastly, an **inner cortex** with starch grains. On the sides of the large lacunae in the middle cortex there are numerous fine **cluster crystals** of Calcium oxalate, each surrounded by a delicate cell wall. The **endodermis** has smaller cells than the other cortical cells immediately external to it and is easily distinguished. It is difficult, however, to make out any clear pericycle. The **vascular bundles** can be picked out by their groups of **vessels,** but they are not clearly marked off from the medullary tissue. The **phloem** shows a few largish sieve tubes, but a considerable part of both xylem and phloem is parenchymatous. There may be a slight development of cambium. There is much less reduction of structure here than in the previous type, *Elodea*.

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[1] Phloeoterma. This name is applied to a cell-layer in the position of an endodermis, *i.e.*, bounding the exterior of the stele, but lacking the anatomical characters of a true endodermis, as is usually the case in stems.

Hippuris vulgaris (Mare's Tail).—Transverse section of stem; stain with Aniline chloride.

On submerged portions of the stem the **epidermis** consists of square cells, without any thickening of the walls, and without any overlying cuticle. In aerial internodes, on the contrary, both outer and inner walls are thickened, though the radial walls remain thin.

The **cortex** consists of a network of large air spaces, separated by parenchymatous membranes, one cell thick. The cells contain some chloroplasts and are thin-walled. The internal air spaces thus formed are important to a water plant which is partially or wholly submerged.

In the centre of the section lies the **stele,** surrounded by a well-marked **endodermis.** There is no pericycle. Within the endodermis is a band of thick-walled **phloem** forming a continuous ring and surrounding a similarly continuous ring of **xylem** in which the slightly lignified vessel walls can be picked out by their yellow colour reaction with Aniline chloride. In the centre is the parenchymatous **pith.** No separate vascular bundles are formed, all the vascular tissue being concentrated into two rings.

***Potamogeton natans*.**—Transverse section of stem; stain with Aniline chloride.

There is a very slight epidermal **cuticle,** diminishing in amount at greater depths below the water. The cortex is **lacunar,** that is, it is broken up into a network of thin sheets of cells arranged vertically, surrounding large air spaces as in *Hippuris*.

The **stele** is small in comparison with the diameter of the stem, and has an **endodermis** of small cells on which the **Casparian band** is well shown. Inside this comes a small-celled **pericycle.** Scattered in the ground tissue of the stele is a small number (six to twelve) of **vascular bundles,** each of which consists principally of a mixture of large and small cells with very thin walls, mostly apparently **phloem** in nature. There are also intercellular cavities (cf. *Elodea*) and one or two **xylem vessels** with relatively slight lignification. Both cortex and conjunctive tissue often contain starch. There are numerous **leaf-trace bundles** in the cortex. Here and there the cortex spaces are traversed by **horizontal diaphragms** of cells with very small intercellular spaces which permit the circulation of air but prevent the passage of water which may accidentally gain entry, as its surface tension is too great to allow its passage through such small apertures. Similar safeguards may be found in most of the other lacunar stems described here.

Juncus effusus (Rush).—Transverse section of the aerial stem.

This leafless aerial stem shows, in transverse section, an external lattice of thickened cells, in the strands of which are embedded the vascular bundles, and the interspaces of which are filled up with soft, thin-walled, water-storage **hydrome.** The surface of the stem is marked with longitudinal ribs which correspond to sclerenchyma strands running below the epidermis and dividing the areas of assimilatory tissue into vertical blocks. This assimilatory tissue consists of layers of very small **chlorenchyma** with no vestige of either palisade or lacunar mesophyll structure, but provided with stomata in the epidermis.

The **pith** is the most remarkable and indeed the unique feature of the anatomy. Its cells have an average of from six to eight arms radiating from a central body. The arms of neighbouring cells are joined at their tips, with a cross wall at the junction, while between them are air spaces which make the whole tissue extremely light and spongy, so that it was used at one time, dipped in tallow, as a primitive candle called a " rush light."

These stellate cells are really double, the central body being vertically elongated with a ring of arms at each end. They apparently arise through differential growth of different regions of the cell wall, the portions which are not attached to neighbouring cells expanding more rapidly and thus becoming invaginated as they increase in area. This inward folding of parts of the cell wall is compensated by the elongation of the arms themselves, so that there is probably little change in the volume of the original parenchyma cell during this peculiar process of development.

3. HALOPHYTES

Salicornia stricta (Glasswort).—Transverse section of the stem; stain with Aniline chloride.

Most members of the Chenopodiaceae have anomalous anatomical structures, and the present example, *Salicornia*, which is also a **halophyte,** growing on marine mud flats, is a striking type.

The stem appears to be succulent, but this is due to decurrent succulent leaf-bases fused to the stem and ensheathing the vascular tissues.

In a transverse section of a well-matured stem there is a broad zone, apparently a cortex, but really leaf tissue, with several layers of **chlorenchyma** externally, the remainder being large-celled **hydrome.** In this tissue are numerous small vascular bundles which make up the vascular system of the adnate leaf. They originate from the stele at the node above, and run, branching downwards, to end blindly in the tissues.

The stele is separated from the leaves by an **endodermis,** the middle lamella of its outer walls being cuticularized (as are some of the radial walls also) and forming thereby an impermeable separating layer. Inside this lies a rather weakly developed **pericycle** and a **cambium.** Primary cortex is absent, and the primary phloem is either missing or represented by a few parenchyma cells only.

On its inner side the cambium produces a solid mass of lignified prosenchyma with here and there small isolated groups of **vessels.** Where the latter are formed the cambium also produces, on its outer side, a few true **phloem** cells. Thus there is at such points a true collateral bundle. But the cambium which lies between the vessels and their associated phloem soon ceases its activity, while the neighbouring cambium continues active. The result is that these tiny collateral bundles are soon left behind, embedded in the growing mass of woody tissue. In old stems it may be seen that these embedded bundles form two or three concentric zones.

4. ETIOLATION

Vicia faba (Broad Bean).—Cut transverse sections of the internode from a normal stem and from one which has been grown from the seed in total darkness (see also Part IX, p. 433); stain in Aniline chloride.

In comparing the two sections notice the presence of **chloroplasts** in the outer tissues of the normal type and their absence in the etiolated plant. The epidermis in the etiolated plant is devoid of cuticle, and the cortex cells are rather larger than in the normal case. There is also somewhat less xylem in the etiolated form, and its fibre cells are rather thinner walled. On the whole, the histological differences are not strongly marked, and are rather quantitative than qualitative.

The question whether a true endodermis is present in the etiolated stem is still undecided.

C. ROOTS

Beta maritima or ***B. vulgaris*** (Beet).—Multiple Cambium Rings. Transverse section of the root; stain in Aniline chloride and mount in Glycerine.

Everyone is familiar with the concentric zonation seen in slices of beetroot. The same thing occurs in the wild Sea Beet, whose root is preferable for sectioning to that of the cultivated Beet. Sections of either will show, however, that the zonation is due to concentric rings of **vascular bundles.** These arise from successive temporary **cambium rings,** each of which forms a ring of independent bundles, of xylem and phloem, separated tangentially by broad **medullary rays,** and radially by intervening bands of parenchymatous ground tissue. The xylem of each bundle is mixed, containing numerous vessels and in each case one narrow tangential band of fibrous **tracheids,** as if the cambium had only actively produced such cells for a short time and had then continued the formation of vessels and xylem parenchyma. In older roots the outer zones of bundles have no vessels or xylem parenchyma, but their xylem consists of a mass of fibrous tracheids only, the later formed cambia being evidently less active than the earlier ones.

Towards the centre of the root the course of the bundles becomes irregular and confused, nor is it easy to distinguish the original primary xylems except in very young roots.

Crocus aureus or ***Gladiolus primulinus*****.**—Contractile Roots. Tangential longitudinal sections of the cortex.

Either of these types will do to illustrate the phenomenon of contractile roots which are produced by many bulbous or cormous plants. In *Crocus* and *Gladiolus* new corms are formed in the spring of each year from axillary buds which lie near the *top* of the old corm and at the expense of its reserves of food. The old corm then shrivels and the new corms are thus necessarily left higher in the soil than the old one. This is adjusted by sending out one or more large and fleshy tap roots from the base of the new corms. These roots penetrate the soil for a depth of several inches without branching. They then shorten and mechanically pull down the new corms into the place vacated by the decay of the old one. In young corms of *Gladiolus* particularly, these contractile roots are of surprising size and strength, being often considerably broader at their bases than the corm from which they spring.

Anatomically the contraction appears to be brought about by the collapse of transverse zones of cortical cells which lose their turgor pressure and are compressed by the neighbouring cells. The structure is, however, not easy to observe, and is not recommended for class study.

Section 2. Climbers

A. TWINERS

1. EXTERNAL MORPHOLOGY

Phaseolus multiflorus (Scarlet Runner Bean).

The stem twines **anti-clockwise** and is assisted in clinging to a support by the numerous, minute, **hooked hairs** with which the stem is clothed. The circumnutation of the apex produces a spiral twisting of the axis, even in the young untwined internodes, as may be seen by examination of the striations on the surface (see *Calystegia*, p. 392).

The position of the leaves on the twining stem is regulated by movements of the **pulvini,** swollen joint-like portions, which may be seen at the base of each petiole and at the base of each individual leaflet of the compound leaf.

Humulus lupulus (Hop).

The stem twists **clockwise,** with a large zone of curvature. The internodes are very long, the pairs of leaves being set widely distant from each other. The stem is beset with many **hooks** (see p. 393) which aid the plant in climbing. The growth of the shoot is very rapid, and the method of twining can be easily seen even in a shoot cut off and placed in a jar of water.

2. ANATOMY

Calystegia sepium (Bindweed).—Transverse section of the stem; stain in Aniline chloride and mount in Glycerine.

The stem, as a whole, twists **anti-clockwise,** but it undergoes a double twist: one, a plagiotropic movement round the supporting object, and the other a nutational twist of the stem on its own axis, which is shown by the spiral direction of the ridges on the stem.

In transverse section the stem anatomy is noteworthy for the following points: (*a*) the large diameter of the conducting vessels; (*b*) the abundance of cortical starch; and (*c*) the presence of **two phloems,** one external and the other internal to the xylem ring. The phloem shows sieve plates which are nearly as conspicuous as those of *Cucurbita.*

There is a weak cambial growth, but very little secondary thickening takes place.

Lonicera periclymenum (Honeysuckle).—Transverse section of stem.

The main difference between this example and *Humulus* lies in the direction of its rotation, which is **anti-clockwise.**

The stem anatomy shows the following points of interest in transverse section: (*a*) the development of only a narrow zone of secondary xylem in the actively twining portion of the shoot, with a high percentage of strongly thickened walls, thus combining strength and flexibility; (*b*) the presence of a remarkable **pericycle** formed of a continuous zone of exceptionally large cells with lignified walls which exceed in diameter even the vessels of the xylem. This may be interpreted as an additional means of toughening the stem against fracture; (*c*) the presence of **chloroplasts** in the phloem, a very rare occurrence; (*d*) the development of the cork cambium at the outer edge of the pericycle.

Clematis vitalba (Traveller's Joy).—Transverse section of a young stem (see also p. 394).

The section is **lobed,** owing to ridges on the stem surface. In each lobe is a large vascular bundle with particularly large cells. The **xylem** is somewhat V-shaped, with one protoxylem, and the metaxylem vessels are very conspicuous. Half-formed vessels may often be seen, next to the cambium, and in process of being differentiated from it. The **cambium** is broad and abundant, with rectangular cells. The **phloem** is a domed mass of cells, sub-equal in size. There is a broad zone of **fibres** in the cortex, outside which, in each ridge, is a mass of collenchyma with thickened angles in the cells.

Between each pair of large bundles is a small bundle identical in structure with the larger ones and situated opposite the grooves on the stem surface.

Cucurbita pepo (Vegetable Marrow) may be used as another type of climbing stem, which shows the characteristic features well developed. The anatomy of the stem of *Cucurbita* is fully described under Part IV, page 199.

Cobaea scandens.—Transverse section of stem (see also p. 394).

The structure is characteristic of a climber. There is a small, parenchymatous, central **pith** surrounded by a small number of **primary bundles** each consisting mainly of unusually large **xylem** vessels. The secondary wood consists of extremely large xylem vessels arranged irregularly in a tissue of xylem parenchyma. The **phloem** is poorly developed outside the secondary xylem and consists of only a few rows of small regular cells. The **cortex** is parenchymatous, consisting of rather flat elongated cells. No medullary rays are developed.

The great size of the secondary xylem as compared with the phloem and the very large size of the secondary xylem elements are characteristic of a climbing stem where large quantities of water have to be transported for long distances through the plant.

B. HOOK CLIMBERS

Galium aparine (Cleavers).

There are two kinds of **hooked hairs,** formed on the stem and on the leaves respectively. Both are helpful to the plant in aid of its scrambling habit.

The first type is formed on the angles of the square stem and consists of a single cell with a rounded venter, like a flask, and a sharply drawn out neck, which is curved throughout. The whole appearance is rather like that of a Chianti bottle with a curved neck. The second type is produced on the upper surface of the leaves. It has a narrow base and a straight columnar body with a curved sickle-like apex.

Both types have thickened walls and the tips are sharp and heavily thickened so that they can act as anchors. The cytoplasm is abundant and the nucleus is easily seen in each case. These hairs may, therefore, be used as demonstration types, if available, to show the appearance of living cytoplasm in an uncoloured cell.

Rubus fruticosus (Blackberry).

The **hooks** or prickles of this straggling plant are apparently lignified and hypertrophied hairs. They " emerge " indiscriminately from all points of the surface and bear no constant relation to any other organ. They cannot, therefore, be otherwise categorised than as **trichomes,** a class of superficial structures (including all the true hairs, surface glands, etc.) which show all gradations from unicellular outgrowths of a single epidermal cell, to the large woody structures here described. They are often intermixed on the surface with true hairs and in some species of Blackberry and in *Rosa spinosissima*, the Burnet Rose, there may be seen all grades of intermediate structures between hairs and prickles.

C. TENDRIL CLIMBERS

Pisum sativum (Garden Pea).

Peas climb by means of **tendrils,** which are modified leaflets. The compound-pinnate leaf is reduced to one pair of leaflets only, the upper pair and the terminal leaflet being changed into thread-like structures, tendrils, which are contact-sensitive and act as attachment organs. There is also a pair of unusually large and leaf-like **stipules,** which no doubt compensate to some extent for the suppression of the upper leaflets of the leaf.

Mutisia ilicifolia.

In this garden climber the leaf blade is small and the midrib has been prolonged into a long simple **tendril.**

The same arrangement may also be seen in *Lathyus aphaca* (Yellow Vetchling), one of the rarer British plants, in which the leaf blade has disappeared altogether and is replaced by the enlarged stipules.

Cobaea scandens.

This climber combines both **tendril** and **petiolar holdfasts.** The leaf is pinnate compound, usually with two pairs of leaflets, and the distal portion of the rachis, together with the terminal leaflets, have been transformed into a compound tendril, the ultimate branches of which bear small clinging hooks. The primary attachment to a support is the work of this tendril, but the petiole takes part in the coiling movement, which shortens the tendril and brings the plant close to the support. The whole leaf may therefore be said to function as a tendril.

Clematis vitalba (Travellers' Joy).

This common plant of calcareous soils climbs by means of **specialised leaf petioles.** The leaf is compound, with three leaflets, and both the main petiole and the petioles of the leaflets are touch-sensitive and coil themselves loosely round objects with which they are in contact, thus acting as holdfasts.

Vitis inconstans (Ampelopsis veitchii).—Virginia Creeper.

The **tendrils** of Virginia Creeper are, morphologically, the terminations of the main stems, the apices of which become converted into these dwarf shoots, bearing only minute scale leaves. From the axil of the leaf immediately below there springs a strong **axillary shoot** which carries on the growth of the stem and soon pushes the tendril to one side, where it appears to be a lateral outgrowth *opposite* to the leaf which subtends the new growing stem. The tendrils themselves branch in a similar way, so that each naked branch of the tendril appears to stand opposite to (*not* axillary to) a scale leaf.

These tendrils, instead of twining round a support, form a small **adhesive pad** at each tip with which they attach themselves to a solid support. The pads flatten out into discs in contact with their supports, and as the central part of the disc shrinks slightly away from the support, while the edge remains firmly attached, the suctional force of a vacuum is thus added to the power of the adhesive.

Additional examples of branch tendrils originating in a similar fashion to that of *Ampelopsis* may be seen in *Cucurbita*, *Bryonia* or *Vitis vinifera*, the main difference in these cases being that the tendrils curl round objects for support and do not develop the peculiar adhesive pads.

Passiflora coerulea (Passion-flower).

These are **branch tendrils,** the morphological nature of which is more obvious than in *Vitis*, because they are lateral structures arising directly from the axils of the leaves.

These branch tendrils, like others of similar nature, extend much beyond the leaves and both they and the parent stem show a fairly rapid circumnutation period (thirty to sixty minutes) which increases the chances of meeting with suitable supports. The tips of the tendrils are curled and easily catch on any twig or shoot with which they make contact. The contact stimulus causes the tendril to twine round the support, forming several clasping coils. The basal portion of the tendril then coils itself into a close helix which acts like a spring in securing the climber against breakage of the tendrils. Sometimes this helix is double, one half of the tendril twisting clockwise and the other half in the reverse direction, with a change-over point half-way along.

Section 3. Epiphytes

Vanda teres or other species of epiphytic Orchid. Root Velamen. Transverse section of aerial root.

The external surface of the aerial roots of an epiphytic Orchid is white, papery and apparently dead. Bunches of such roots are produced adventitiously from the lower sides of the stem and hang down, free and unbranched, for lengths varying from a few inches to more than a foot. If one of these roots be wetted with a drop of water on the end of a finger, it will be seen that the water is readily absorbed by the surface, while the imbibed region becomes darker and the green colour of the internal tissue shows through. If the root is broken across, the fresh green tissue of the core is visible, surrounded by a colourless covering.

This covering tissue is called **velamen.** It consists of several layers of empty parenchymatous cells, the walls of which are strengthened by a network of fine **cuticular ridges.** The cell walls are so thin that water is readily absorbed by the tissue, like a sponge ; thus it takes the place of root hairs as an absorptive layer under the exposed conditions of an aerial root. Furthermore most, if not all, of the cell walls are perforated by large open pores. At the inner boundary of the velamen there is a single layer of highly thickened cells, the **exodermis,** which protects the living tissues within by resisting water evaporation. At the same time it provides for the passage of liquid water inwards, through a number of unthickened living cells, which occur at intervals, known as **passage cells.**

Within the exodermis is the ordinary **root-cortex** and the **stele.** The latter is of normal monocotyledonous type, except that the thickened **endodermis** is discontinuous, being interrupted at a number of points by groups of thin-walled cells, with only a Casparian band on their walls.

Section 4. Parasites

A. STEM TYPES

Cuscuta epithymum or other species (Dodder).

The Dodder is a fairly common parasite on Heather, on Gorse and on Clover. It has delicate, shining, reddish or yellowish stems which look like a skein of coloured sewing silk tangled up with the branches of the host plant. Closer inspection will show that the stems are leafless and devoid even of scales, but bear small fascicles of attractive pinkish flowers like miniature *Convolvulus*. The parasite is attached to the host by minute adventitious roots, like the attachment roots of Ivy, which penetrate the host tissues and attach themselves to the vascular bundles of its stem. They are called **haustoria.** The Dodder stems twine closely round the host, the haustoria being formed on the inner side of the curls.

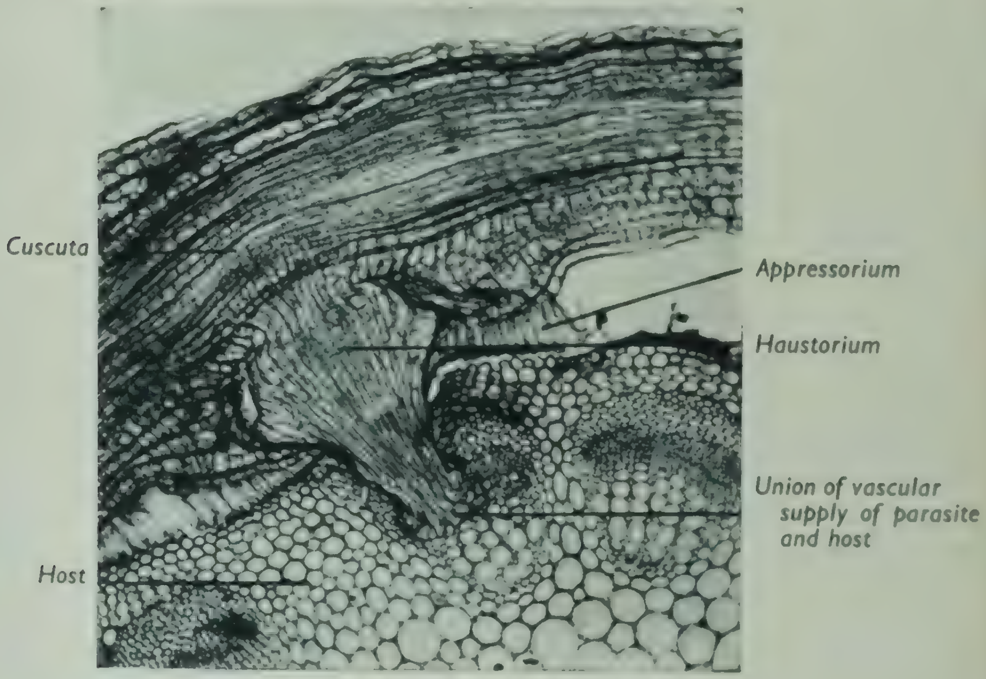

FIG. 107.—*Cuscuta epithymum*, on *Trifolium*. Section through host to show invasion.

A section transverse to the host stem at the level of one of these curls will be more or less longitudinal to the *Cuscuta*, and should show several haustoria (Fig. 107). Round each is developed a suctorial disc or **appressorium,** which attaches itself adhesively and very firmly to the cuticle of the host. This gives the needful holdfast which enables the point of the haustorium, which originates endogenously, to force itself into the host tissues. Each haustorium has a single central vascular bundle, and the phloem of this attaches itself to the host phloem, the xylem to the host xylem in the cambial region, thus tapping the flow of nutritive fluids.

Instances have been known in which the *Cuscuta* haustoria penetrated the tissues of the parasite itself instead of the host, thus becoming self-parasitic.

Viscum album (Mistletoe).

The Mistletoe is attached to the branches of the host tree (Poplar, Apple, Lime, etc.) by woody ingrowths which are the product of the primary root of the seedling. This rootlet penetrates as far as the wood and there fixes itself permanently, so that it is gradually enveloped by the increasing thickness of the wood of the host, in annual rings, as years pass. Meanwhile, it lengthens by means of a basal meristem, so that its growth keeps pace with that of the wood. From this basal meristem the primary root (or **sinker**) produces laterals which run longitudinally under the bark of the host and send down additional sinkers themselves. Those nearest the base of the Mistletoe stem are those first formed, and hence are the oldest.

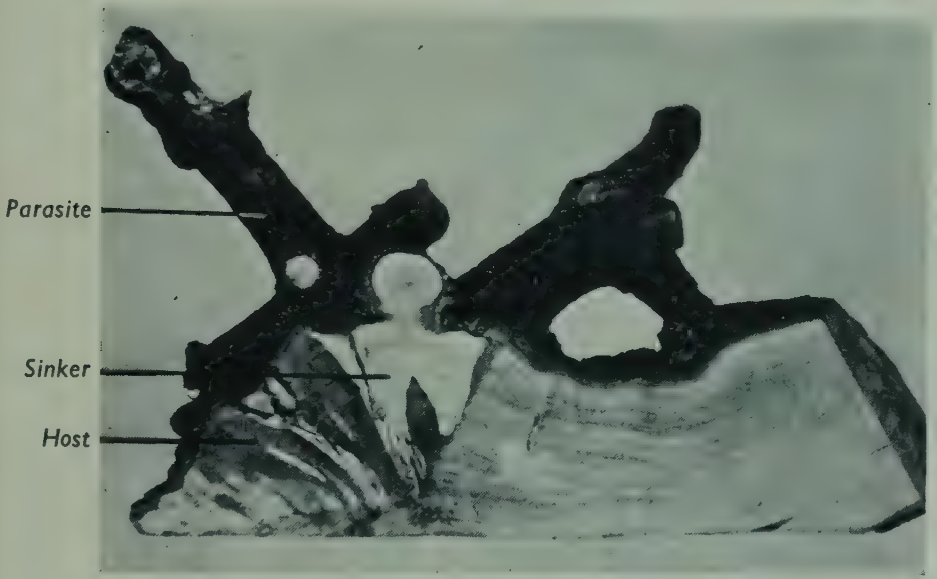

FIG. 108.—*Viscum album.* Section through host to show sinkers.

The sinkers become woody and can be well seen in sections cut (preferably longitudinally) through the branch and the base of attachment of the Mistletoe plant (Fig. 108). They do not actually penetrate the wood already formed, but are engulfed by its subsequent growth in thickness, so that they appear to have grown into it. The lateral cortical roots can also form **adventitious buds** which break outwards through the bark and produce new leafy shoots (*i.e.*, " suckers ").

As *Viscum* is not only furnished with its own green leaves, but is, moreover, evergreen, it can carry out photosynthesis at all times and takes nothing but water and inorganic salts from the host tree. It is therefore classed as a semi-parasite.

B. ROOT TYPES

Orobanche apiculata (*O. minor*) or other species (Broom-rape).

The Lesser Broom-rape does not occur on Broom, but chiefly on Clover, some badly infested fields of which may yield hundreds of specimens of the parasite.

Orobanche attaches itself to the roots of the host and forms a rounded woody tuber, sessile on the host's roots. This tuber is the direct outcome of the swelling up of the primary root of the seedling *Orobanche*, after it has succeeded in attaching itself parasitically. For some time this constitutes the whole plant. Then (during the first year in *O. minor*, or after several years in *O. major*) an adventitious bud is formed on the tuber, thickly covered with brown scale leaves, which develops into the aerial inflorescence. After flowering the parasite usually dies.

Lathraea squamaria (Toothwort).

Lathraea is parasitic on the roots of several native trees, notably Elm and Hazel. Like *Orobanche* its stem is underground, attached to one of the larger roots of the host, but unlike the latter, the stem is elongated and freely branched and bears closely set, decussate, fleshy leaves of a dull creamy tint with an internal cavity (see below). Kerner says that plants weighing up to 10 lb. are by no means rare. Attachment to the host is carried out by **haustoria,** which are adventitious roots, as in *Cuscuta*, but these are longer and branched, resembling the tendrils of Virginia Creeper.

From the ends of the branches arise the inflorescences, while lateral buds carry on the growth of the stem. The inflorescences come up into the light and bear spikes of dull purple flowers on whitish stems which have a very curious and striking appearance.

The Leaf.—Longitudinal sections through the leaf show that its structure is entirely parenchymatous, with very large isolated starch grains which show the attached **leucoplasts** remarkably well (see Part II, Section 2, p. 41). There is an irregular central cavity, which has a small opening on the lower side of the leaf near the base. The sides of this cavity are lined with small **glandular hairs,** each with a four-celled head. The structure is suggestive of the trap of an insectivore, and certainly remains of small insects may sometimes be found in the leaf cavity. It has not been proved, however, that *Lathraea* really digests these insects or absorbs their soluble constituents, though it is probable that it does at least the latter.

Pedicularis sylvatica (Lousewort).

This is one of a group of semi-parasites, not uncommon in grassland, which attach themselves in the early stages of growth to the roots of grasses by means of **haustoria.** Later on these attachments are usually lost and the plants live independently, but may cause considerable damage to grasses in the spring. Roots of the parasite which make contact with a grass root swell up to form a minute cushion of tissue, the **appressorium,** partly enveloping the grass root. From the centre an outgrowth of large elongated cells presses into the grass root, the tissues of which become disorganized. There does not appear to be any vascular penetration of the host plant.

C. ALGAL TYPES

Polysiphonia fastigiata.

This species usually occurs growing on the Brown Seaweed, *Ascophyllum nodosum*, and is common around the British coast. The *Polysiphonia* is regarded as a semi-parasite, since it normally occurs on *Ascophyllum*, or very rarely on *Fucus serratus* or *F. vesiculosus*. Sometimes it may be found growing independently on the rocks.

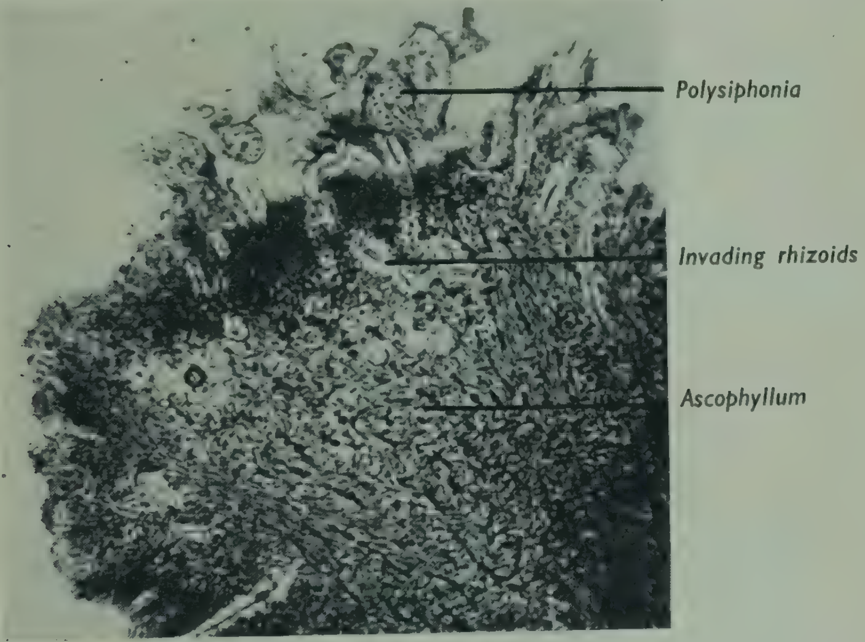

FIG. 109.—*Polysiphonia fastigiata.* Section to show the invasion of the brown algal tissue by the red alga.

Cut sections through the *Ascophyllum* thalus at the point where the tuft of *Polysiphonia* filaments is attached, treat with Vanillin in strong Hydrochloric acid[1] and mount in glycerine.

The rhizoids of *Polysiphonia* will be seen to penetrate deeply into the cortical tissue of the *Ascophyllum* (Fig. 109) and, at the tip, the wall of the rhizoid is relatively thin and contains considerable quantities of granular cytoplasm. This suggests that the rhizoids are really **haustoria** absorbing food from the *Ascophyllum*. This interpretation is further emphasised by a study of the staining reaction. *Ascophyllum nodosum* does not contain starch in a form usually found, but the cells will be seen to stain red when treated as above. In the region where the rhizoids occur it will be observed, however, that the *Ascophyllum* cells do not stain red, indicating that they are devoid of starch, which has been removed by the haustoria of the *Polysiphonia*.

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[1] 0·5 gm. Vanillin, in 0·5 c.c. Alcohol, 0·5 c.c. water and 3·0 c.c. of concentrated Hydrochloric acid.

Since, however, *Polysiphonia fastigiata* is sometimes found growing freely in the absence of *Ascophyllum nodosum*, it can obviously synthesize its own food, though it is noticeable that under such conditions the vegetative growth is less vigorous.

We have here, then, an example of an Alga which can live independently, but grows more vigorously when some of its food is supplied by another Alga, and we may regard that as an intermediate example between epiphytism, which is exhibited by many of the smaller seaweeds which grow attached to larger Algae, and complete parasitism, as in *Choreocolax polysiphoniae* and *Harveyella mirabilis*, two members of the Rhodophyceae, neither of which can live independent lives.

Epiphytism among seaweeds can be well seen by studying the collection of species which may be found attached to the holdfast and stipe of any of the larger Laminarias. In this case the small Algae apparently obtain no food from the plant to which they are attached, and merely occur there because the tissues provide protective lodgment for the reproductive bodies after they have been liberated into the sea.

Section 5. Symbiosis and Special Modes of Life

A. LICHENS

Peltigera canina.—Transverse sections of thallus and apothecium.

This is one of the largest of the common Lichens, and its broad leathery fronds are frequently to be seen among Mosses on banks or in woods. Closely allied species may also often be found on old sandhills near the coast. The fronds are about 1 in. across, forming patches several inches wide, which are bluish-green in colour when wet and brownish when dry. The plant is prostrate and close growing, the upper surface usually slightly wrinkled and with upturned edges showing a light-coloured under-surface with abundant **rhizinae** or rooting fibrils.

The reproductive organs are **apothecia,** like those of the Ascomycetes, and they are formed on small upright lobes at the edge of the thallus, bearing on their upper sides a smooth dark-brown **hymenium**. The apothecia are large, even for so large a Lichen, and are often nearly 1 cm. across.

The whole plant looks so distinctive and differs so markedly from anything in other groups that it is difficult to realize that it is only a compound of a Fungus and an Alga. This, however, is clearly seen if a section of the thallus is examined. The upper surface is formed of a **cortex** of fungal cells closely pressed together into a " false tissue " (**plectenchyma**). Below this is a layer containing abundant cells of the filamentous Blue-green Alga *Nostoc*, interwoven with fungal **hyphae** which are connected upwards with the cortex, while below the algal zone they branch out to form a loose spongy **hypothallus.** The algal zone is still often called the " gonidial zone " from the obsolete idea that the Alga was a reproductive organ (gonidium) of the Fungus.

A section through the apothecium of *Peltigera* shows that the **hymenium layer** is composed of a very large number of delicate vertical filaments, closely packed together, like the pile of velvet. These are called the **paraphyses,** and it is their upper ends which form the brown surface of the apothecium. Hidden among them are numerous, broader, cigar-shaped cells, the **asci,** in which are the **ascospores.** The ascus contains a bundle of eight spores, which are filamentous, composed of four cells each, and nearly as long as the ascus itself. Such an arrangement is quite typical of the order Pezizales among the Ascomycetes and shows that we are dealing here, not with a peculiar organism, but with an Ascomycete Fungus living in association with an Alga. The components can be separated and both cultivated independently, but under these circumstances the Fungus forms no spores.

Xanthoria parietina.—Transverse sections of thallus and apothecium.

The common orange-coloured Lichen of old roofs and walls. The body of this Lichen is thalloid, like that of *Peltigera*, but smaller and more delicate. It adheres more or less closely to the stone, though the margins are free and are divided into rounded lobes. The reproductive organs are formed in **apothecia,** which are circular saucer-shaped structures raised above the general level of the thallus and furnished with a thick rim. The apothecia are usually the same colour as the thallus and are crowded together in groups on the older parts near the centre of the plant. In section, the thallus is seen to consist of an upper **cortex,** three or four " cells " thick, the cells in this case (as in *Peltigera*) being merely sections of tightly packed filaments or **hyphae.** This type of tissue is called **plectenchyma.** Immediately below this come the algal cells, or **gonidia,** in a scattered layer. The inner tissue, or **medulla,** is composed of very loosely woven hyphae, and the lower surface is again formed by a narrow cortex layer.

Sections through an apothecium (Fig. 110) show that the lower part and the margin are formed of the same tissues that compose the thallus, with scattered gonidia. The upper surface shows a **hymenium** composed of sterile **paraphyses** and of **asci.** The paraphyses are narrow and difficult to see separately, they are packed so closely together, but the asci are broader and are easily recognized by the **ascospores** they contain. These spores are **polarilocular,** that is to say, the contents are chiefly in two spaces, one at each end of the spore, connected by a narrow channel. The ascospores are the reproductive bodies of the fungal constituent only. When they germinate a new Lichen can only be synthesized if the Fungus meets with the appropriate Alga (*Cystococcus humicola*), which is, however, very abundantly distributed.

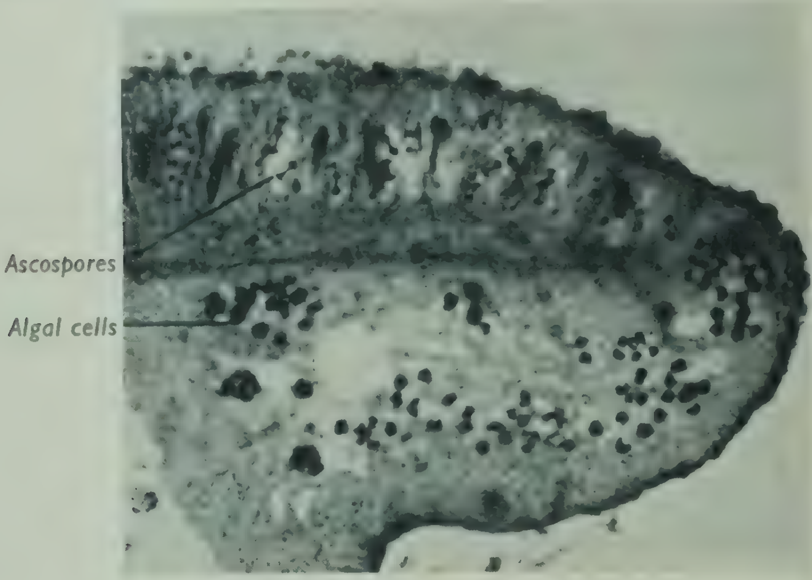

FIG. 110.—*Xanthoria parietina.* Vertical section through the edge of an apothecium.

B. MYCORRHIZA

Neottia nidus-avis (Bird's Nest Orchid).—Transverse sections of the root; stained with Iodine or Lactophenol Cotton Blue.

Neottia is not a very common plant in this country, but it is not infrequent in woods on calcareous soils, especially in shady and humus-laden spots, so that material can often be obtained. It is one of the very best examples of **endotrophic mycorrhiza,** and so is included for this purpose.

Transverse sections of the roots clearly show a zone in the outer cortex, three to four cells broad, in which every cell appears to be densely filled with a tangled **mycelium,** in the midst of which the cell nucleus may be seen when stained.

As an alternative, the finest rootlets of *Calluna vulgaris* (Ling) may be used, if mounted whole in Chloral-iodine or Eau de Javelle to clear the tissues, but one is not so certain to find the endophyte in this case as in *Neottia.*

Another alternative is the rhizome of *Monotropa hypopitys,* which sometimes turns up in similar situations to *Neottia,* though it also occurs in old sand-dune hollows.

C. ROOT NODULES

Trifolium sp. (Clover).

Dig up a plant of any species of Clover or Trefoil and wash the roots carefully. They will be seen to be studded with small nodules. These are not due to any disease, but are developed at the points invaded by the Nitrogen-fixing Bacteria of the soil, whose activities help to provide the host plant with Nitrogen compounds. This symbiosis makes plants of this type valuable sources of Nitrogen for the soil, so that Clover has a definite part in the course of the rotation of crops, replacing, at least in part, the Nitrogen taken from the soil by cereals.

The Bacteria invade the young root through the root hairs, and the nodules are developed by hypertrophy of the root cortex.

Nitrogen-fixing Bacteria.—Cut through a large root nodule with a razor and dab the cut surface gently on the centre of a perfectly clean slide. Nodules on Bean or Lupin roots are larger, and may be preferred for this purpose to those of Clover.

Allow the smear to dry and then heat it gently over a Bunsen flame or by passing it three times rapidly *through* the flame, so that it feels hot when placed on the back of the hand, *but not too hot to bear*. This heating fixes the organisms to the slide, but it is most important not to overheat.

When the slide is cool, stain the film with a drop of Carbol Fuchsin or of Methylene Blue for two or three minutes. Wash the stain off gently with water and blot the slide dry by careful pressure with a hard filter paper. When quite dry mount in Balsam.

Examine under the high power of the microscope, using the highest-powered eyepiece you have, which should be about × 10. A bright light is essential.

The Bacteria appear as very minute stained rods, immensely numerous and distributed both singly and in groups. The rods are very variable in length, some being mere dots. This is due to the successive transverse divisions by which the cells multiply. The length of the individual, therefore, depends on the length of time which has elapsed since the last division. The form of the rods is also somewhat variable and some branched Y-shaped forms will be seen.

D. CARNIVOROUS PLANTS

1. ***Utricularia vulgaris*** (Bladderwort).

The plant is a submerged aquatic, found floating in the still water of ponds and ditches, especially in fairly clear water in peaty districts, *i.e.*, water poor in soluble nutrient materials. Only the yellow flowers are raised above the water surface.

The stems of *Utricularia* are long and delicate and the leaves are filamentous and branched, while attached to them, or sometimes in place of them, are the **insect-catching bladders,** which may, therefore, be regarded as modified leaves or portions of leaves.

Mount a small bladder in water and observe that it has a **mouth** at the opposite end to the stalk, and that this mouth is surrounded by stiff branched hairs. The mouth is closed by a **door** or **flap,** but its complex structure cannot be made out properly without the aid of sections (see Fig. 111). The lower edge of the flap rests against a minute ridge of tissue in such a way that its inward movement is impeded by friction. The interior of the bladder is lined with glands, which take the form of groups of four hair-like cells with a common base. These can be seen in a bladder dissected open. They absorb water vigorously, producing a negative pressure inside the bladder and sucking inwards the side walls. Nothing more happens, however, unless one of the **trigger hairs,** growing on the flap itself, is touched, whereupon the flap snaps inwards, followed by a rush of water bearing with it any small objects in the neighbourhood. As the walls expand into the original rounded shape the flap drops back into place, with an action not unlike that of a bent piece of card or celluloid held in the fingers, which can be made to snap one way or the other by very slight movements of the muscles.

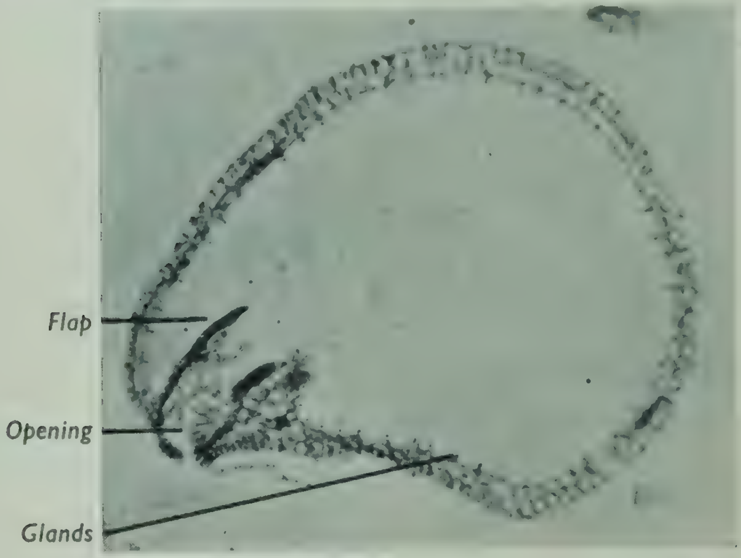

FIG. 111.—*Utricularia vulgaris.* Vertical section through the trap.

So successful are these traps that a single plant may capture several hundred Copepods, *Daphnia*, etc., in a short space of time. No digestive enzyme has been yet demonstrated inside the bladders. The insects apparently die slowly by asphyxiation and may probably be decomposed by bacterial action, their soluble products being absorbed by the hair glands mentioned above.

2. *Nepenthes destillatoria, N. villosa, etc.* (Pitcher Plant).

This genus of tropical epiphytes is confined to the Old World and is fairly widely distributed in rain forests. What appear to be long leathery leaves are really **phyllodes,** *i.e.*, petioles flattened into a lamina which takes the place of the true leaf lamina. The **pitchers** are produced at the ends of these phyllodes and are simply specialized parts of them, while the true leaf lamina is modified into a small " lid," or more correctly, an " umbrella " held over the open mouth of the pitcher (see Fig. 112).

The pitchers are rendered attractive to flies by the rather lurid coloration in splotches of purple and crimson. On the upper part of the inside surface there are numerous microscopic **glands** in the form of minute round cushions (Fig. 113) which secrete a watery solution containing Pepsin, partly filling the pitcher. Flies enter the pitcher and drown in the fluid in such large numbers that the pitchers are sometimes robbed by birds. The nitrogenous compounds in the insects' bodies are hydrolysed by the Pepsin, and when thus rendered soluble are absorbed by the glands on the pitcher walls.

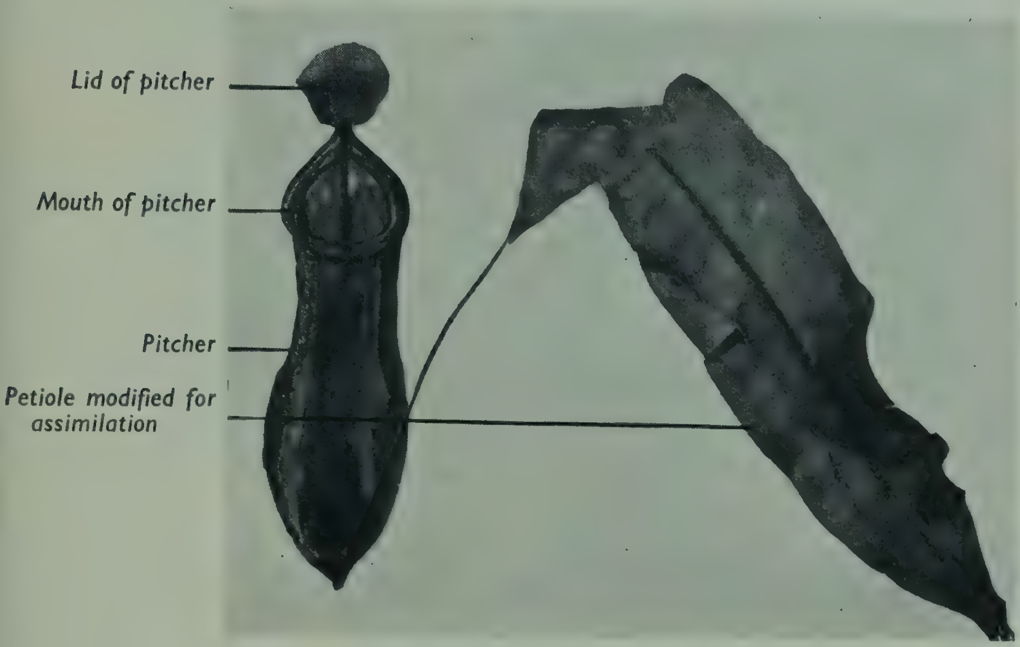
Lid of pitcher
Mouth of pitcher
Pitcher
Petiole modified for assimilation

FIG. 112.—*Nepenthes.* A single leaf to show the parts.

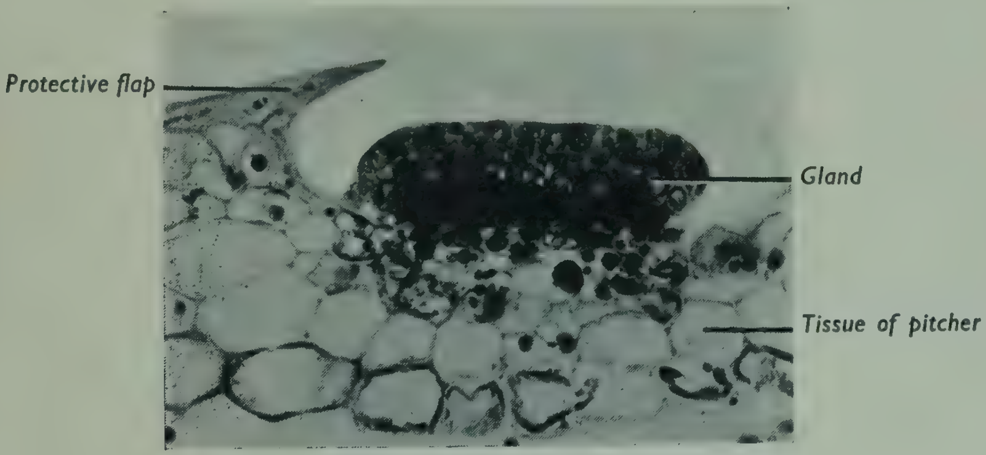
Protective flap
Gland
Tissue of pitcher

FIG. 113.—*Nepenthes.* Vertical section through a pitcher gland.

Everything is done to facilitate the insects' descent to Avernus. The margin of the pitcher is smooth and polished, and is furnished with teeth curving inwards and downwards, much as in a lobster pot, while the inner surface is covered with smooth downwardly directed flaps which cover over the glandular cushions, making an insect's descent easy but seriously hindering any attempt to return.

3. ***Pinguicula vulgaris*** (Butterwort).

The plant is common in boggy peaty places, especially among *Sphagnum* moss. It is recognizable by its star-like rosette of pale yellowish-green leaves, 1 to 2 in. long, with curled-up edges. The leaf surface looks slimy and feels slightly sticky, and small flies are easily caught by the mucilage secreted. The surface is covered by microscopic **hair-glands** which secrete the sticky juice. These glands are of two kinds, with short and long stalk cells respectively, and it is supposed that the digestive juice is secreted by one set and the products of digestion absorbed by the other, but this is not clearly established. Contact of a nitrogenous body with the leaf surfaces stimulates a copious discharge of digestive juice, accompanied by a further inrolling of the leaf margins, which form a trough to contain the juice. Digestion is rapid, and when complete it is followed by the drying up of the glands and consequently of the leaf surface. The remains of the prey are then easily carried off by the wind or the rain, after which the leaf is again bathed with mucilage and functions once more as a trap.

4. ***Drosera rotundifolia*** (Sundew).

The plant gets both its Latin and its English names from the tiny drops of dew-like secretion with which the hairs of the leaf are crowned. It forms little reddish rosettes, with the leaves closely pressed against the wet peaty soil in which it usually grows. The plant is common in western and northern districts, but scarcer in the eastern counties.

Each leaf consists of a petiole and a circular green lamina. The lamina is, however, covered with long red "tentacles," which are modified hairs, each tipped with a round **gland** which secretes a drop of watery mucilage.

Cut off a few hairs from a leaf and warm them in Eau de Javelle for a few minutes. Mount in Glycerine. The glands will be seen to have a solid core of lignified **xylem tracheids,** and a thin strand of tracheids is continued down the stalk and joins on to the vascular system of the leaf. The gland also has two covering layers, a surface layer of secretory cells of irregular shape with thickish side walls, and an under layer of tangentially flattened cells. On the stalk there are occasional degenerate **stomata** without a pore, recognizable only by the shape of the guard cells.

The **hair-tentacles** are sensitive to touch and respond by bending inwards, so that any small insect caught by the leaf is gradually smothered and finally digested by the accumulated secretion of all the hairs together (see also pp. 234 and 377).

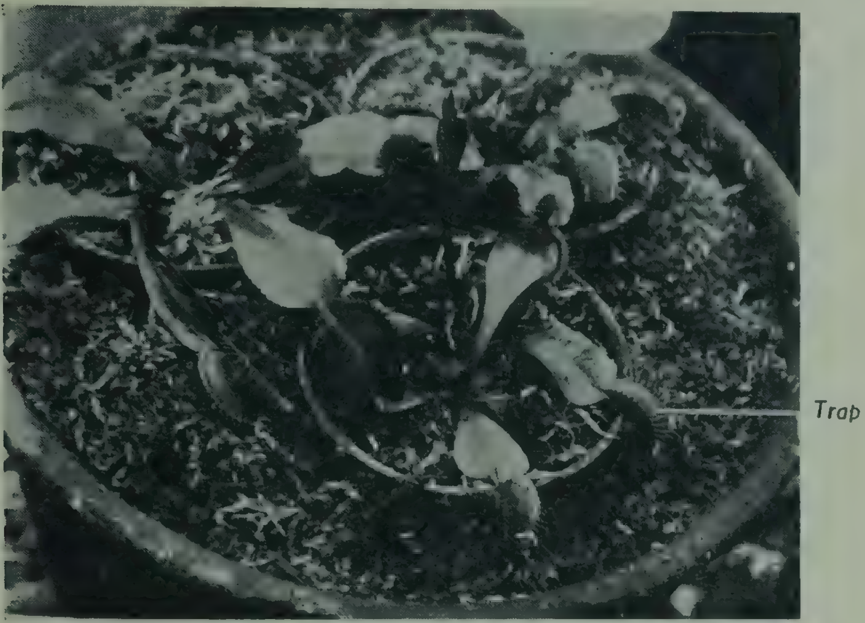

FIG. 114.—*Dionaea muscipula.* A plant showing the modified leaves.

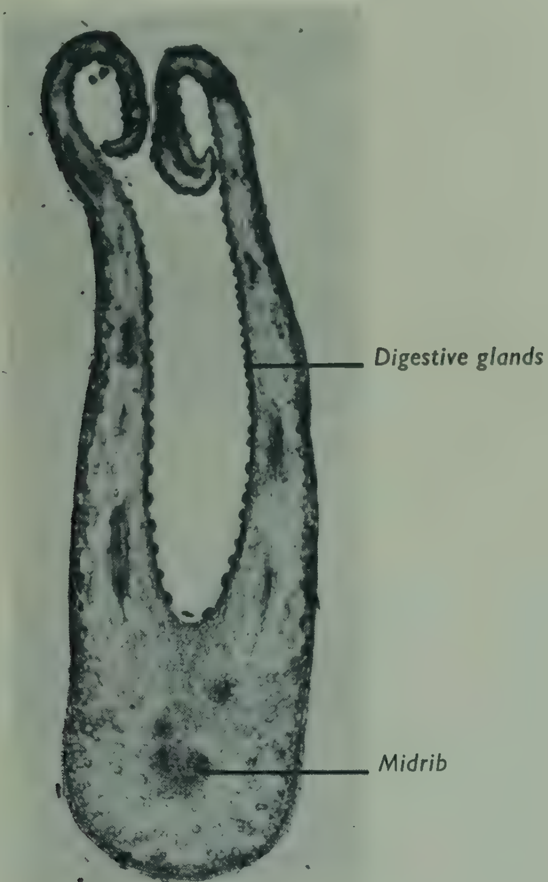
Digestive glands
Midrib

FIG. 115.—*Dionaea muscipula.* Vertical section through the leaf.

5. ***Dionaea muscipula*** (Venus' Fly Trap).

This is a swamp-loving plant native only to the southern United States. The leaves are about 2 to 3 in. long and form a rosette on the surface of the ground (Fig. 114). Each leaf has a broad **phyllodic petiole** and a **lamina** which is formed of two halves, hinged together at the midrib like the pages of a book so that they can open and close. The edge of each half is armed with stiff **spines,** which interlock like the teeth of a rat trap, when the lamina is closed up (see Fig. 115).

The upper surface of the lamina is also beset with sparse **hairs,** three on each segment, of a stiff appearance and a few millimetres long. A vascular bundle of the lamina ends at the base of each of these hairs. The hairs are obliquely set, and in the angle between each of them and the leaf surface is a group of thin-walled cells distended with water.

The hairs are exceedingly sensitive to touch, for when one is pressed down it closes on the thin-walled cells like a nutcracker and drives water from them into the vascular bundle below, thus setting up a wave of hydraulic pressure in the vascular system, which conveys the stimulus throughout the leaf.

The result of this disturbance is to cause the two halves of the lamina to close suddenly, owing to the disturbance of turgor pressure in the cells at the midrib. The movement takes only ten to twenty seconds, and is rapid enough to trap any insect which is wandering over the leaf. The leaf surface itself is also sensitive to touch, but the reaction is much slower.

An interesting point about these sensitive leaves is that they do not react to water drops falling on the sensitive parts. Thus they are saved from the inconvenience of frequent reaction to rainfall.

Section 6. Vegetative Propagation

A. NATURAL PROPAGATION

1. STOLONS

Helianthus rigidus (Perennial Sunflower).

The term **stolon** is used to indicate a short, thin, lateral (axillary) shoot, given off below the ground, at the base of an upright leafy shoot. Its growth is limited to one season, and in the following year its apex turns up vertically to form another leafy shoot, which in its turn produces underground stolons. It differs, therefore, from a **runner,** which lies above the ground, and a **rhizome,** which is a perennial underground stem, often the main stem of the plant. Similarly, a stolon which is sufficiently fleshy would be classed as a **tuber.** These terms have, however, often been confused, and intermediate cases do occur which show that the differences are more those of degree than of kind.

The perennial Sunflower of gardens "*Harpalium*" produces regular crops of stolons such as are here defined, several from the basal nodes of each flowering stem. They are slightly tuberous towards their turned-up apices and are marked with scale leaves or their leaf scars. They are provided with adventitious roots and are, therefore, potentially independent of the parent plant. They afford a remarkably effective way of spreading densely over a limited area.

Alternatives: *Mentha viridis*, etc. (Garden Mint), *Aster novae-angliae* (Michaelmas Daisy) and *Linaria repens* (Creeping Toadflax). These are all good alternative examples of this class of vegetative reproduction.

2. SUCKERS

Rubus idaeus (Raspberry).

The underground system consists of **rhizomes,** which turn up vertically at their apices to form the upright flowering " canes," and of woody **roots,** growing horizontally, on which buds arise endogenously and also form new canes. The young buds show as small white outgrowths on the brown roots. They are called **suckers.** This formation of adventitious buds on true roots (not rhizomes) is uncommon, but other good examples are Poplar, especially White Poplar (*Populus alba*), and the Hedgerow Elm (*Ulmus procera*), which form abundant suckers, especially after the main tree is felled.

3. OFFSETS

Sempervivum tectorum (Houseleek).

The original rosette of leaves sends off short side shoots of a peculiar type which are called **offsets**, and are frequently found in leaf succulents of similar habit of growth to the Houseleek. They are branches, axillary to certain of the lower leaves, forming thin shoots about 1 in. long, which bear only a few very reduced scale leaves. At the end of each such shoot is formed a secondary rosette of closely placed succulent leaves, which is a miniature of the main rosette. From its base adventitious roots appear, making the branch potentially independent. The thin shoots are easily separated from the mother plant, and the offset may then fall or roll away and establish itself separately. Often, however, they remain to form a cluster of secondary rosettes round the principal central plant.

4. BULBILS

Festuca ovina var. vivipara.

Sheep's Fescue grass growing in alpine grassland at over 2,000 ft. altitude in this country has almost entirely abandoned the formation of flowers for the production of vegetative **bulbils.** These replace the flowers on the inflorescences, one bulbil replacing one normal spikelet

(Fig. 116). Each consists of a minute vegetative shoot, from the base of which adventitious roots emerge. They are detached by means of an abscission layer.

This habit, which confers the advantage of time-saving on these alpine plants, whose growing season is so short, is a fixed character and continues even under garden culture. The form may be looked upon, therefore, as a mutation from the type of the species. A similar bulbiferous form of *Poa bulbosa* is also known, which grows at sea level and has no alpine associations. The rare *Poa alpina* is, however, both alpine and frequently bulbiferous. So is *Polygonum viviparum*. The habit is, therefore, apparently advantageous in an alpine environment.

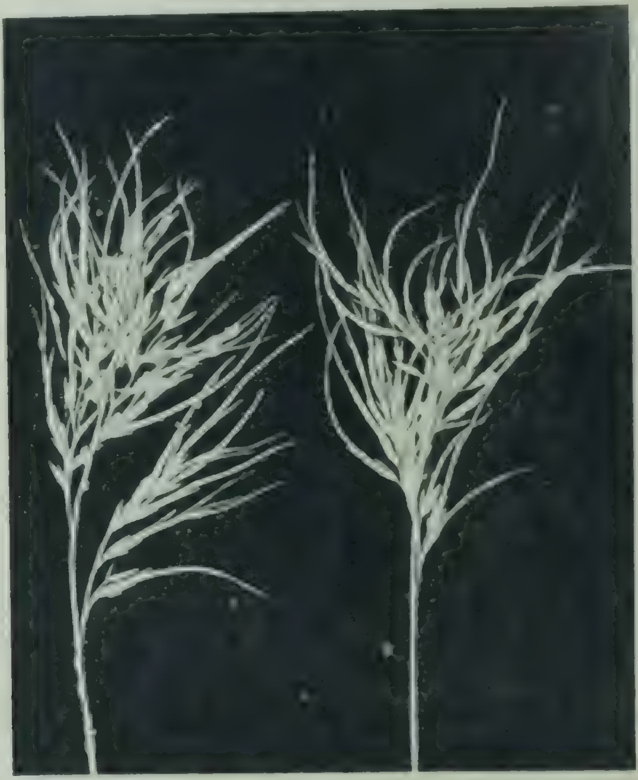

Fig. 116.—*Festuca vivipara*. Flower head showing the young plants developing in place of flowers.

Allium cepa var. bulbiferum (Tree Onion).

Several species and varieties of *Allium* form inflorescences in which the flowers are partly or wholly replaced by **bulbils,** *i.e.*, by vegetative shoots. In the variety of Onion, called by gardeners the Tree Onion, these bulbils are nearly 1 in. in diameter and can be used for food. An inflorescence pedicel is formed which grows to the usual height of about 3 ft. and bears terminally an umbel of about a score of these small bulbs, each of which commences the development of foliage leaves before being detached, after which adventitious roots are formed and independent life continues.

Asplenium bulbiferum.

This Fern used to be very commonly grown as a pot plant for room decoration, and is still frequently seen. Its peculiarity is that there are numerous **adventitious buds** produced on the surface of the frond, which develop leaves up to 2 in. long while attached to the parent plant.

These buds or **bulbils** are not automatically detached like those of *Bryophyllum* (see p. 412), but continue to grow on the leaf until, in course of time, the latter is shed, when the buds produce adventitious roots in contact with the damp soil and thus establish themselves independently.

There is a race of the common *Cardamine pratensis* (Milkmaids) which bears adventitious buds similar to the above at the base of its leaflets on the rachis.

If specimens of *Lilium bulbiferum* or of *Dentaria* (*Cardamine*) *bulbifera* can be examined they will show the transformation of the normal axillary buds into bulbils, which are detached like the other examples cited. The former species is frequently cultivated, and the latter may be found wild in the south-eastern counties of England.

5. LAYERS

Rubus fruticosus (Blackberry).

Layering is a common gardening practice. Lateral shoots near the ground are bent down and pegged on, or just under, the soil surface, often with an incision cut in them. They root adventitiously and may then be detached for transplanting. This process is often carried out by nature in wild plants and very commonly in Blackberry. Examination of any thriving bush will show some arching shoots which have bent down until their tips reach the ground. There they often bury themselves in the soil and send out many adventitious roots, while the apex turns upwards once more and begins a new shoot. Such **layers** may remain for long attached to the parent plant, whose rooting area is thus greatly increased year by year, or they may become detached and continue their existence as independent centres of growth.

6. HIBERNACULA

Elodea canadensis (Canadian Pondweed).

Plants of this species collected in late September or early October will usually have **hibernacula** formed on the ends of many of the branches. These take the form of dense aggregations of leaves, forming something like a much enlarged apical bud, but, of course, without any specialized bud scales.

These hibernacula are detached by an abscission layer across the stem and sink to the bottom of the water, where they remain in the mud until spring, when they produce adventitious roots at the base and develop into new plants.

Hydrocharis morsus-ranae (Frogbit).

Although it is a much less common plant than *Elodea*, the Frogbit may often be found in ponds and ditches, except in the extreme north of England or in Scotland.

It grows luxuriantly and covers the water with matted rhizomes and tufts of leaves like those of a tiny water lily.

In early October the plant will be found to have produced short branches from the base of each leaf tuft, terminating in closed buds of oval shape about ½ in. in length. These are the **turions,** a form of hibernacula or winter buds. They are detached, like those of *Elodea*, and pass through the winter at the bottom of the water. The rest of the plant meanwhile dies off, so that next season's plants will be produced by the growth of the turions. A longitudinal section through a turion shows the rudiments of adventitious roots already formed. The turion is enclosed externally by two overlapping scale leaves and has many small scale leaves within. These scale leaves are **stipules,** and from between them grow out in spring the new foliage leaves which since the previous autumn have been tightly rolled up between the scale leaves.

The sinking of hibernacula in winter is due to the heavy accumulation of storage starch in their tissues, which can easily be seen in sections, and their refloating in spring is due to the formation of gas bubbles between the scales, produced by assimilation and respiration, principally the former. These act as floats to bring the turion to the surface.

7. ADVENTITIOUS BUDS

Bryophyllum calycinum or other related species.

The vegetative reproduction of this plant by detached buds is sufficiently striking to have made it an object of popular interest. It is a fleshy plant, and the succulent leaves have a number of minute notches round the margins. At the base of each notch is a small group of cells which remain meristematic, while all around them are becoming differentiated into leaf tissues. In some species of *Bryophyllum* these cells remain dormant so long as the leaf is attached to the parent plant and only develop after it has been isolated. This peculiarity has given to much interesting experiment and speculation regarding the control of the individual over its constituent parts. In other species, however, development regularly takes place on the growing leaf. This development produces a small bud consisting of a rosette of several minute rounded leaves and a tiny bunch of fine reddish rootlets adventitiously produced. This plantlet is detached by the formation of an abscission layer at its base, and rapidly establishes itself as an independent individual (Fig. 117).

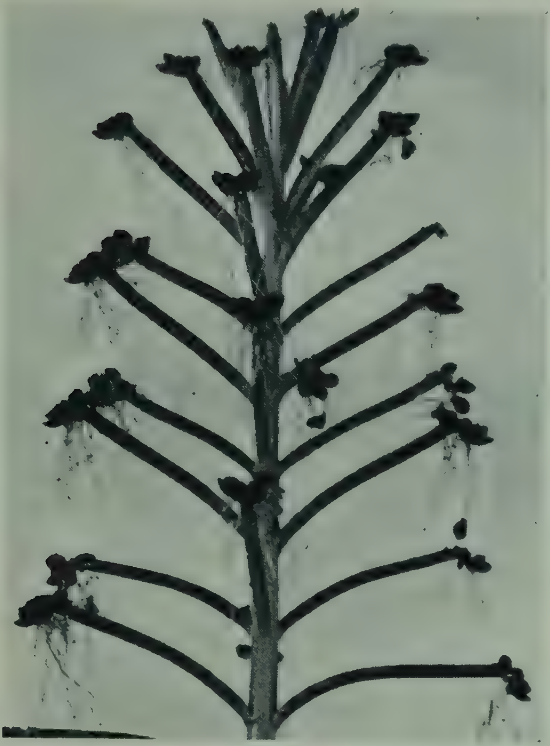

FIG. 117.—*Bryophyllum tubiflorum.* Young plants growing from the tips of the leaves.

B. ARTIFICIAL PROPAGATION

1. STEM CUTTINGS

Salix spp. (Willow).—See also Part VII, page 369.

Most woody plants can be grown from cuttings of the young wood, sometimes with a " heel " of older wood attached. Few, however, are easier than Willow in this respect. Pieces of Willow stem, cut from young branches, will root and grow if one end is inserted in soil or will even do so if merely suspended over water in a jar so as to be in saturated air.

Roots develop adventitiously from the lower nodes, *i.e.*, those inserted in the soil, while the upper buds grow into new shoots.

Try Willow cuttings standing in water in a covered jar, so that they are completely enclosed. Place some in the water right way up and others upside down. After a couple of weeks it will be seen that the upside-down shoots are developing roots at the **morphologically lower end,** which is, however, **uppermost** in the jar, and shoots from the buds which are lowest. This is a fine example of inherent **polarity,** which is far from being fully understood.

2. ROOT CUTTINGS

Crambe maritima (Seakale).

This plant grows so freely from **root cuttings** that it is difficult to eradicate it from a place where it is established, for the smallest portion of root is able to develop into a new plant by means of adventitious budding.

Dig up part of the root from an old Seakale plant. Cut several short lengths and plant them upright under the soil, both ways up. They will show the same fixed polarity as Willow cuttings. If thin slices, less than 2 mm. thick, are planted, they produce roots from one end only, but shoot buds from both ends.

Temperature has a marked effect on controlling polarity, though, as we have just seen, gravity is not strong enough. If the ends of cuttings are suspended in warm water, which can be kept warmed continuously by a small gas jet with a thermostatic control, the warmed end will produce stem buds, whether it is the lower or the upper end morphologically. A very small temperature difference (2° C.) is enough.

The **callus** from which the buds develop comes from the cambium. It is interesting to note that if the cambium is severed in a piece of Seakale root the latter regenerates as if it were two distinct pieces, although all the tissues except the cambium may be intact. Several experiments of this kind may suggest themselves, and they are very easy to carry out.

3. CALLUS FORMATION

Taraxacum officinale (Dandelion).

The roots are good material for showing **callus** formation. Cut the root into pieces about 1½ in. long. Wash the latex thoroughly off the surface with cold water. Place the pieces on moist filter paper in a Petri dish in the light. Callus appears on the second or third day on the cut surfaces in a ring just outside the woody zone. It becomes green in five or six days and begins to develop leaf primordia in one week. These develop into foliage leaves, while the callus enlarges to cover the whole of the cut surface.

PART NINE

PLANT ECOLOGY

Introductory Remarks on Field Work

In addition to laboratory studies every student of Botany should, as a matter of course, carry out a certain amount of field work to which the laboratory studies should be related. Where an extended study away from the laboratory is contemplated, arrangements must be made for certain necessary equipment to be taken into the field.

For field work every student should be provided with a **field notebook.** This book should open like a reporter's notebook. It should measure about 6 in. by 4 in. and consist of alternating sheets of ruled and of graph paper. It should be fitted with an elastic band and a pencil, and should be provided with a string loop so that it can be worn round the neck. Every student should also possess a good-quality lens which, when in use, is best attached to the student by a string.

A small **flora,** such as Hayward's " Botanists' Pocket Book," will enable all higher plants, except very critical species, to be determined in the field.

For survey work it is best to divide students into a number of small **groups,** each employed in a particular part of the survey work. While the number and composition of the groups must depend on the number of students taking part, not more than three persons can be profitably employed together in mapping or levelling a given area, while two are sufficient to carry out a valence analysis. When a number of separate surveys are contemplated on successive days, it is an easy matter to adjust the rotation of groups, so that each student performs each section of the work at least once.

In **selecting an area** for ecological investigation great care should be exercised to avoid taking in too much to complete in the time available, with the result that skimping is encouraged. Base lines of more than 100 to 150 ft. length are rarely needed, and it will seldom be found necessary to make transects longer than this, if due care is given to the selection of the site in advance. Time taken in the selection of the site and the planning of the work before the survey is started will save much time later on. Not only the area but the various plant communities represented on it must be carefully determined beforehand, as it is the distribution of these communities which is the object of the survey.

It will be found that the work ties together, so that records obtained by one group can be confirmed by those of other groups. For example the levels should agree in general terms with the changes in the plant communities, but more accurate checking can be made with profile charts and belt transects. Changes in the composition of the vegetation brought out by these surveys should agree with the observations on the map. A path, with no ground flora, should appear in a transect at the same point as it is shown on the map. The results of Valence analysis and the associated Covering Capacity and Frequency should bring out the same analytical results as those revealed by belt and profile charts for the same area.

The careful examination of the charts produced during a field class provides useful work in the laboratory at a subsequent date. Finally, if permanent corner posts can be driven into the ground to mark the area of the survey, a return to the same site after an interval of a few years and a repetition of the survey will provide very interesting comparative data.

Practical ecological work may be divided into the following sections :—

1. Survey work, including sociological analysis.
2. Soil studies.
3. Physiological observations.

The description of the methods employed in survey work are given in detail in our book "Practical Field Ecology"[1] and, since they would involve long and elaborate illustrated descriptions, they will not be repeated here, where we are concerned with laboratory studies. The above book also describes field methods of soil analysis. These have been omitted in the present book but alternative methods more suitable for laboratories have been described. These methods in general require either heat or apparatus which could not easily be taken into the field. The physiological observations given in this book are more extensive than those described in "Practical Field Ecology," but there is some overlapping in this section.

Section 1. Soil Studies

A. ANALYSIS OF SOILS

1. SAMPLING

Before any work can be done on the analysis of soils a **good sample** must be obtained.

In most soils the surface-growing plants or litter must be cleared away, together with 1 or 2 in. of semi-decayed material from the surface, and the trowel is then driven in cleanly to remove a portion between the depths of 2 and 6 in. Repeat this in about half a dozen different spots over the area and mix approximately equal samples from each. The aggregate sample may then be used for analysis. If the sample is to be removed to the laboratory, or for any other reason must be kept some time before use, it should be packed in an air-tight tin lined with waxed paper. A field box to hold twenty-four soil tins (4 oz. size) is a valuable adjunct to the field survey equipment.

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[1] "Practical Field Ecology," by R. C. McLean and W. R. Ivimey Cook. Published by Messrs G. Allen & Unwin Ltd. Second Edition, 1950.

2. MECHANICAL ANALYSIS

This is a process for separating the various grades of particles of which the mineral part of a soil is composed. These are classified by international agreement into the following categories :—

Larger than 2 mm. = Gravel.
2 to 0·2 mm. = Coarse Sand.
0·2 to 0·02 mm. = Fine Sand.
0·02 to 0·002 mm. = Silt.
Below 0·002 mm. = Clay, *i.e.*, the colloidal fraction of the soil matter.

Examples.—Percentages, exclusive of gravel (after G. W. Robinson) :—

	Coarse Sand.	Fine Sand.	Silt.	Clay.	Organic Matter.	Calcium Carbonate.
Sandy Soil . .	66·6	17·8	5·6	8·5	1·5	*Nil.*
Heavy Loam .	13·6	17·3	24·7	35·1	9·2	*Nil.*
Heavy Clay .	0·9	7·1	21·4	65·8	2·8	2·0

It will be seen that such an analysis gives concrete expression to the idea of **texture** in a soil, which is of the greatest importance agriculturally.

Method.—Take about 30 gm. of air-dry soil and rub it down with the wooden end of a pestle till all lumps and crumbs of soil have been reduced. Weigh out 20 gm. of this soil. Pass through a 2 mm. sieve. Weigh the residue on the sieve. This is **gravel.** Place the remainder in a large beaker and add 500 c.c. of "20 vol." Hydrogen peroxide. Heat gently on a water bath and stir while bubbling. After thirty minutes add another 25 c.c. of H_2O_2 and heat again for fifteen minutes. Remove, add 100 c.c. of 1 per cent. Hydrochloric acid. Boil for about a minute. Cool and filter. Wash on the filter with hot water. (Dry and weigh. This treatment removes all the oxidizable organic matter generally called **humus,** also the carbonates, which should be separately estimated, see p. 422. The loss of weight of the treated soil therefore equals **humus + carbonates.** These cause cohesion of soil particles, and so must be removed as a preliminary to the mechanical analysis.)[1]

Wash the wet or dried residue off the filter paper on to a sieve of 0·2 mm. mesh. If this is unobtainable use one of $\frac{1}{100}$ in. mesh, which is very nearly correct. The sieve must be placed over a large beaker to collect everything which is washed through it. Wash the material on the sieve thoroughly. Dry and weigh. This represents the **coarse sand.**

Make up the value of the washings in the beaker to about 500 c.c. Add a little dilute Ammonia or 2 c.c. of normal Sodium hydroxide and transfer to a large bottle. This must be shaken mechanically for several hours, preferably for about twelve hours, to break down all aggregations of particles. Transfer to a tall cylinder and make up the volume to 1 litre with water. Shake vigorously by hand and stand for *exactly* four minutes forty-eight seconds. Take a 20 c.c. pipette and at the end of the above time remove 20 c.c. of the suspension as rapidly as possible from a depth of 10 cm.

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[1] The operation in brackets may be omitted if separate estimations of humus and carbonates are to be done later.

The pipette should be fixed through a hole in a large cork, or a piece of wood, which rests on the top of the cylinder with the tip of the pipette placed at the correct depth in the fluid. Place the 20 c.c. thus withdrawn in a weighed evaporating dish. Dry and weigh. The weight of the residue, in milligrams, divided by 4, gives the percentage of **silt + clay.**

Shake the cylinder again, and this time let it stand for exactly eight hours. Take another 20 c.c. sample as before. Evaporate and weigh. The weight of the residue in milligrams divided by 4 gives the percentage of **clay,** alone.

Subtracting this percentage from the former gives the percentage of **silt,** alone.

Empty the cylinder into a litre jar or beaker. Make up with water to a depth of 10 cm. Stir and stand for four minutes forty-eight seconds exactly. Pour off quickly all the turbid liquid and repeat the process until the water poured off is quite clear. Collect the remaining sediment, dry and weigh. This is **fine sand.**

The values for the Clay and Silt are direct percentages. The other figures must be calculated to percentages of the original weight of air-dry soil.

3. MICROSCOPIC EXAMINATION

Pass a little air-dry soil through a $\frac{1}{100}$ in. mesh sieve and mix a very small quantity with a drop of Glycerine on a slide. Examine by transmitted light from below and also by reflected light from above. Mineral particles of every shape and size make up the greater part of the soil. Most of these are transparent or translucent and represent crystal fragments of quartz, felspars, micas and other rock-forming minerals. The humus appears opaque by transmitted and brown by reflected light. It will be noticed that it forms clumps with the small mineral grains, cementing them together. This is a most important characteristic of humus. It tends to " lighten " the soil and the observation explains why it must be removed before mechanical analysis.

B. PHYSICAL PROPERTIES

1. NATURAL MOISTURE CONTENT

This is naturally a very fluctuating quantity, but an average estimate may be sought by taking a sample about twenty-four hours after rain has fallen. More scientific and more instructive, however, is the construction of a graph, based on a number of estimates at different times and seasons, to show the **range of natural variation** in this respect of the soil examined.

Take a sample of about 10 gm. of soil and weigh it in a weighed basin. Dry in an oven at or near 100° C. Cool in a desiccator and weigh. Replace for an hour in the oven. Cool and re-weigh. If no further loss has taken place calculate the loss observed as a percentage of the weight of the oven-dried soil.

Compare at least two soils of different textures in the same way.

2. SATURATION CAPACITY

This is the percentage of water held against gravity by a soil in which the pores are completely filled with water. It serves, therefore, as a measure of Pore Space (see below), and it is a quantity expressive of the relative proportions of coarse and fine grains in the soil, as this controls the packing of the grains together and hence the available pore space.

The measurement is made with a **soil box.** This is made from a piece of metal tubing, or sheet metal formed into a tube, ¾ in. deep by 2 in. diameter. Inside one end of this fits tightly a stout brass wire ring, to support a disc of perforated zinc, cut to fit inside the tube. The perforated bottom is held in place by a second wire ring. Fit a piece of filter paper over the perforated bottom and weigh the whole. Now fill the box with soil which has been thoroughly broken up by sieving through a 1 mm. sieve. Carefully pack the soil a little at a time, tapping the box on the bench to settle it thoroughly, but do not press the soil down. Finally, use a straight edge to strike off the top flush with the edge of the box. Weigh.

Place the box in a flat-bottomed dish with about ½ in. of water and leave it until the soil surface appears thoroughly wetted. Dry the box rapidly on the outside, allow to drain for a few moments, weigh and then place in an oven at 110° C. to dry. Cool in a desiccator when it has attained constant weight, and re-weigh.

Finally, determine the weight of water held by a piece of filter paper similar to the one used in the box. Calculate as follows :—

Weight of box + filter paper $= a$
Weight of box + filter paper + air dry soil $= b$
Weight of box + wet filter paper + wet soil $= c$
Weight of box + dry filter paper + oven dry soil $= d$
Water held by filter paper $= f$

Moisture content of saturated soil, *i.e.*, weight of water (in grams) taken up by 100 gm. oven-dry soil

$$\frac{c-(d+f)}{d-a} \times 100.$$

3. PORE SPACE

The measurements made under 2, above, are required, and in addition the volume of the box in cubic centimetres = V.

The percentage of pore space in the dry soil may then be calculated from the expression :—

$$\frac{(c-b)\times(b-d)}{V}\times 100$$

that is, the weight of water held per unit *volume* of soil. The weight of water in grams and its volume in cubic centimetres are taken as equal for this purpose. Greater accuracy is not required.

4. MOISTURE EQUIVALENT

This has been arbitrarily defined as the percentage of water retained by a soil when subjected to a centrifugal force of 1,000 times gravity. Although an arbitrary quantity it is a useful guide to the relative water-holding powers of different soils. Its direct determination is a matter of some difficulty, but an alternative measure, which is a function of the moisture equivalent, is easily obtained and may be used instead.

Take a small Buchner filtering funnel about 2 in. in diameter and fit it with a hard filter paper. Weigh and then fill it about three-quarters full of sieved (1 mm.) air-dry soil. Weigh again. Fit the funnel into a filtering flask and attach it to a water pump. Fill the funnel with water and apply suction. When all the water has gone through, fill up again and allow the soil to drain, under suction, for ten minutes after the water has disappeared from the soil surface, keeping the funnel covered with a wet cloth during this time. Disconnect from the flask, wipe the funnel dry and weigh again.

Calculate the percentage of water remaining in the soil, adding the percentage present in the original air-dry soil, as determined by an oven-dried sample, and deducting the water held by the filter paper, as in the last two experiments.

5. CAPILLARY POWER

Compare the power of two soils of different texture to raise water by capillary action. Take two lengths (about 2 ft.) of 1 in. glass tubing. Grind one end of each smooth and tie a piece of linen over it. Fill the tubes about three-quarters full of sieved (1 mm.) air-dry soil, using a sandy soil and a heavy loam respectively. Add the soils slowly, tapping all the time to secure settling of the particles.

Place the lower, covered, ends in water and note the rise in the soil columns. The heavier soil raises the water further than the other, but much more slowly, owing to imbibition by the soil colloids.

6. STICKY POINT

Spread out about 10 gm. of 1 mm. sieved air-dry soil on a glass plate and spray it with water from a wash bottle until it is definitely wet and sticky. Work up the mass into a stiff paste with a spatula. Scrape it off the glass and knead in the hands until a point is reached at which the mass no longer spreads, but begins to clean the fingers and form a smooth ball. At this stage it may be cut cleanly with a knife. A sample portion of the ball is weighed and oven dried to determine its water content. Successive determinations should not differ by more than 1 per cent.

In this condition the soil colloids should be fully imbibed and the pore space, reduced to its minimum by the breaking-up of the compound particles, entirely filled with water.

A useful figure for comparing soil textures is **Hardy's Texture Index.** This is the expression: $\text{T.I.} = \text{M} - \frac{\text{S}}{5}$, where M = moisture content at the Sticky Point and S = percentage of sand.

The higher the Texture Index, the heavier the soil. The upper limit is about 55.

7. FLOCCULATION OF CLAY (see also Part VII, p. 294)

The clay fraction of the soil consists of particles of colloidal dimensions which carry a negative electric charge. If positively charged ions are present in excess in the water, these negative charges may be partly or wholly neutralized, with the consequence that the most powerful force holding the particles apart is annulled. The result is flocculation or the formation of aggregate particles, analogous to those which build up the "crumb" structure in the soil, a most desirable attribute from the cultural point of view.

Make a fairly strong suspension (about 2 per cent.) of china clay in distilled water containing a trace of Ammonia and divide it between three test-tubes. To one add 1 c.c. of a 2 per cent. Potassium chloride solution. To the second add 1 c.c. of a 0·2 per cent. solution of Calcium chloride, and to the third add 1 c.c. of decinormal Hydrochloric acid. Potassium is monovalent and has but little flocculating power. Calcium is divalent and has approximately four times the flocculating effect of Potassium. The hydrogen ions in the acid are only monovalent, but they are extremely mobile and consequently are more rapidly effective.

The effect is shown by the time of settling of the suspensions and may be represented by curves, showing the depth of the clear supernatant layer plotted against time.

The effect of varying the amount of the neutralizing ions added may be tried, or, neglecting the time factor, the highest dilution which will cause complete settling may be compared. The power of ions in this respect varies exponentially with their valency, forming a series, x, x^2, x^3, x^4, etc.

The effect of lime in opening the texture of a soil may be shown practically as follows: Cut two discs of perforated Zinc about 2 in. across and place each in a glass funnel. Cover each with a large unfolded filter paper and place about 200 gm. of a wet clay soil in each funnel, spreading it out to make as even a layer as possible. Place each funnel over a measuring cylinder. Fill one funnel with distilled water and the other with lime water. Compare the amount of water percolating through in the same time in each case.

8. PERCOLATION THROUGH SOILS

Get a piece of wide glass tubing (a lamp chimney is good) about 1 ft. long and select a cork to fit it. Bore a ½ in. hole through the cork. Cover the cork with a piece of muslin and press it into one end of the tube, muslin inside, so that the muslin is held in the tube, stretched over the hole in the cork.

Place successively (not simultaneously) in the tube about 100 gm. of fresh sand, of garden loam and of leaf mould respectively. In each case, after filling the tube, hold it over a measure and pour a litre of water through it. Measure the amount of water percolating. The difference between this and 1 litre shows the amount retained by the material in the tube. It will be found that leaf mould retains up to three times as much as the loam and about ten times as much as the sand. This shows that the humus in the soil contributes very importantly to its water-holding capacity.

C. CHEMICAL PROPERTIES

1. SOIL REACTION

Place about 1 in. of fresh soil in a test-tube, add about the same amount of a normal solution of Potassium chloride[1] and shake up. Allow to settle and pipette or decant off some of the clearest part of the fluid into another test-tube.

Another method is to mix an equal quantity, or rather more, of finely precipitated Barium sulphate with the soil before adding water. This carries down the finer soil particles quickly, and although the fluid may remain turbid it is white and does not interfere with the colour of indicators.

Test with a drop of Universal Indicator, which is a mixture with a range from about pH 4·0 to 11·0. This will give the approximate hydrogen ion concentration.

More accurate measures are best made with the " Capillator " (British Drug Houses Ltd., Graham Street, City Road, London, N.1). The indicator and the soil extract are mixed in equal quantities in a small watch-glass and sucked up into a hard glass capillary tube. This is compared with a card of standard capillaries containing the same indicator at a range of different pH. In this way estimates can be made to 0·2 of an integer of pH.

2. CARBONATE CONTENT

This is usually estimated by decomposition with dilute acid and measurement of the Carbon dioxide produced.

One simple but accurate method for this is to join up two filtering flasks, one large and one small, by their side necks. A trap of glass wool in a glass tube should be included in the connection (see Fig. 118).

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[1] Distilled water may be used instead of Potassium chloride, but the results are rather more variable and settling of the suspension is slower.

Each flask is fitted with a rubber stopper through which is passed a tapped separating funnel. The small flask should also be fitted with a short length of glass tubing, either tapped or closed by a rubber tube and clip, to act as an air vent.

Place about 5 gm. of soil, weighed, in the small flask and fill the funnel with Hydrochloric acid. Fill the funnel of the large flask with standardized N/5 Barium hydroxide. Open the air vent and run in, first, the Baryta solution into the large flask and then the Hydrochloric acid on to the soil in the small flask. Close the air vent at once. When bubbling ceases, shake the soil flask and warm gently to drive off Carbon dioxide from solution, while shaking the Baryta flask to promote absorption. Allow the apparatus to stand overnight or for some hours, so that all the Carbon dioxide may be absorbed. Unstopper the large flask, add Phenolphthalein indicator and titrate with N/5 Hydrochloric acid to determine the excess Baryta not precipitated by the Carbon dioxide. Titrate till the pink colour of the indicator is just discharged. Compare the amount of acid needed to titrate an equal quantity of fresh Baryta. Let V = difference between the two

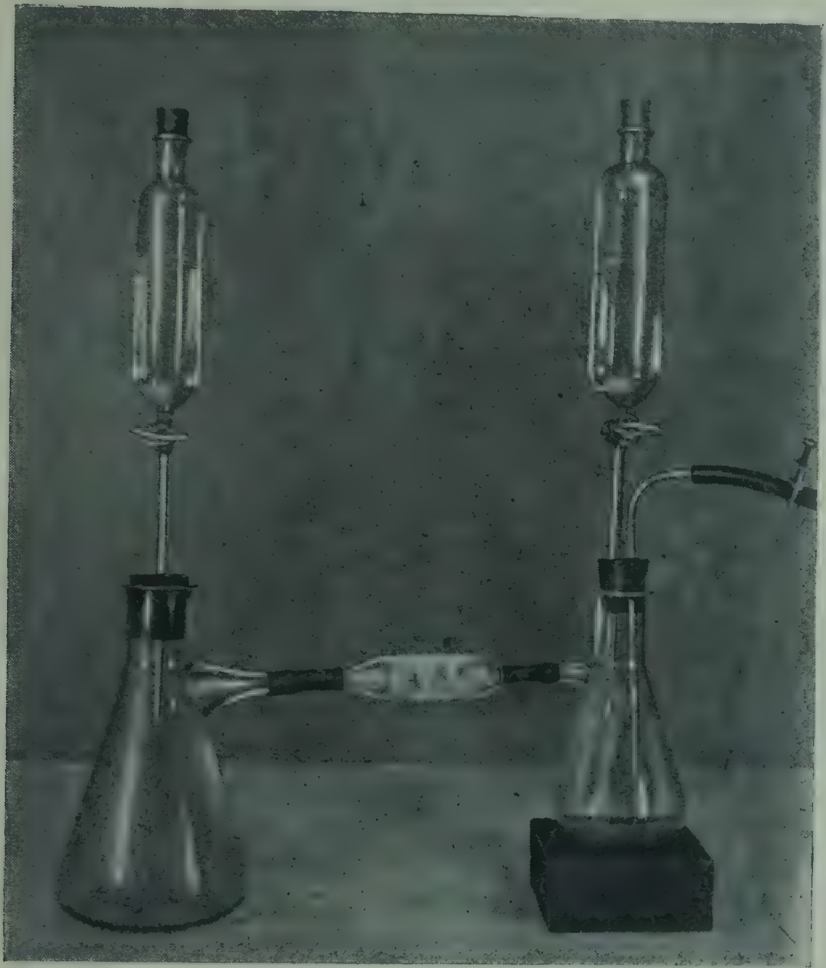

FIG. 118.—Apparatus for determining the Calcium carbonate content of soils.

titrations and $N =$ the normality of acid used (*i.e.*, 0·2), then $V \times N \times 22{\cdot}0 =$ milligrams of Carbon dioxide produced. This is converted to milligrams of Calcium carbonate in the soil sample by multiplying by the factor 2·27. From this the percentage can easily be worked out, by proportion.

3. LIME DEFICIENCY

If the soil shows no reaction with dilute acid it may be tested for its deficiency in bases, *i.e.* (agriculturally), "lime." A convenient, semi-quantitative method, applicable in the field as well as in the laboratory, is **Comber's thiocyanate test.** The reagent is a saturated solution of Ammonium thiocyanate in absolute Alcohol, which is applied to a small quantity of *dry* soil in a test-tube. This is really a test for Ferric ions, and the application is based on the fact that only when there is a deficiency of bases will there be any excess of free Ferric ions to react with the thiocyanate. Thus the deeper the red colour produced the greater the deficiency of base. A modification of this method uses 5 per cent. aqueous Potassium salicylate, which gives a violet colour with Ferric Iron. The colour is less intense than with thiocyanate, but the reagent can be used with wet soil, which is sometimes convenient.

The use of this test as an index of lime deficiency in soils depends on the replacement of adsorbed ions of K, Ca, Fe, etc., from the surface of the soil colloids by NH_4 ions. Soils poor in the monovalent and divalent ions will release Ferric and Aluminium ions. Aluminium thiocyanate is insoluble in Alcohol, but the Ferric thiocyanate will dissolve and produce a red colour.

With pond or bog soils the test may give no colour reaction, although a lime deficiency may nevertheless exist. This is because the reducing conditions in such soils keep the Iron present in the Ferrous state. In such cases it is well to add Hydrogen peroxide after the thiocyanate, one drop to each 10 c.c. of solution in the test-tube. If the red colour now appears for the first time, or if any colour present darkens in tone, it not only shows that a lime deficiency exists but also that reduced Iron is present, which is oxidized to the Ferric state by the peroxide.

4. LIME STATUS AND LIME REQUIREMENT

The **lime status** is the ratio between the amount of exchangeable Calcium held by a soil in its natural condition and the amount it holds when saturated in contact with Calcium carbonate solution. Both the lime status and the **lime requirement,** or the amount of Calcium needed to saturate the natural soil, can be determined by one operation, as described by Puri and Vanshylla. Strictly speaking, the equilibrium between Calcium carbonate and the soil does not represent theoretical saturation, but it is the practical limit.

Take 10 gm. of soil, 50 c.c. of water and about 1 gm. of Calcium carbonate. Mix, and shake periodically for two to three hours or leave overnight. Leave the stopper of the bottle loose for any Carbon dioxide to escape. If the bottle be connected to a water pump and the air pressure in the bottle is reduced, equilibrium will be reached in about thirty minutes.

Cool the mixture to 10° C. and add 50 c.c. of the following solution :—

Potassium oxalate	3·7 gm.
Potassium acetate	9·8 gm.
Potassium carbonate	0·41 gm.
Water	100 c.c.

Shake for about half an hour. Filter through fluted paper and titrate 50 c.c. of the filtrate with standardized Potassium permanganate, after acidifying with Sulphuric acid. The equivalence is : 1 part of permanganate equivalent to 1·39 of oxalate ion.

The decrease of oxalate concentration is equivalent to the exchangeable Calcium in the soil, in equilibrium with Calcium carbonate solution.

If Ca_1 = exchangeable Calcium in the original soil (see Experiment 5 below) and Ca_2 = exchangeable Calcium after treatment with Calcium carbonate, then the Lime Status $= \frac{100\ Ca_1}{Ca_2}$.

The Lime Requirement is $Ca_2 - Ca_1$.

Taking the weight of one acre of soil to 6 in. depth as 2,000,000 lb., the Lime Requirement in acre/tons is $Ca_2 - Ca_1 \times 0{\cdot}28$ of CaO, or $Ca_2 - Ca_1 \times 0{\cdot}5$ of $CaCO_3$.

5. EXCHANGEABLE CALCIUM

This is generally looked upon as equivalent to " available " Calcium in the soil, although this is not strictly correct. Still it is a useful index to the availability of that all-important soil element which forms the chief base available for neutralizing soil acidity.

There are many methods proposed, of which the following, due to Williams, is one of the simplest :

Place 25 gm. of soil in a beaker with 200 c.c. of N/2 Acetic acid. Stir and allow to stand for at least two hours. Filter off the solution into a litre flask and wash the soil on the filter paper with the same acid, about 100 c.c. at a time, until 1,000 c.c. of filtrate have collected in the flask.

Divide the filtrate into two portions of 500 c.c. Keep one aside for the next estimation (Total Exchangeable Bases). To the other portion add 5 c.c. concentrated Ammonia, 10 gm. Ammonium chloride and excess of strong Ammonium oxalate solution, to precipitate all the Calcium as oxalate. Bring to the boil and allow to stand overnight. Filter off the precipitate, wash and dissolve in dilute Sulphuric acid. Titrate this solution with standard Potassium permanganate. The relations are as follows : 1 mg. of $KMnO_4$ is equivalent to 2·31 mg. of crystallized Oxalic acid, and 1 mg. of the latter is equivalent to 0·317 mg. of Calcium. Therefore 1 mg. of permanganate is equivalent to 0·732 mg. of Calcium.

6. TOTAL EXCHANGEABLE BASES

Williams' Method.—Take 500 c.c. of the filtrate from the previous estimation and evaporate. When the liquid is reduced to small volume transfer it to a small evaporating dish before evaporating to dryness. Dry thoroughly in an oven and then ignite at a dull red heat for about five minutes. When cool add 20 c.c. of N/5 Hydrochloric acid and rub the dish with a rubber-shod glass rod (a " Policeman ") until all the deposit is dissolved. Cover and allow to stand overnight. Pour through a small filter paper and wash the filter with boiled distilled water. The excess of acid in the filtrate is titrated with standard Sodium hydroxide, using Phenolphthalein as indicator. Stop when a pink colour is reached, which lasts for about ten seconds on shaking. From this may be calculated the weight of acid neutralized by the soil bases present in the extract, though without an exact knowledge of their chemical constitution and equivalence it is not possible to calculate their total weight. The method is therefore only a comparative one, for which purpose the weight of bases may be calculated as Calcium oxide.

7. PRESENCE OF NITRATES IN THE SOIL

Plug a filter funnel with cotton wool or glass wool and fill the funnel with garden soil. Pour water on the soil and collect the first 1 or 2 c.c. which come through.

To 1 c.c. of the filtrate add 4 c.c. of a solution of 0·1 gm. Diphenylamine or Diphenylbenzidine in 10 c.c. of strong Sulphuric acid. A blue colour shows the presence of nitrates. Nitrates are not adsorbed by the soil, and the above treatment shows that they escape in the soil drainage water. If, however, the amount present is small and no colour is shown by the above treatment, try boiling a sample of soil for half an hour with four times its weight of water. Filter, boil the filtrate and filter again if there is any precipitate. Then test the final filtrate as above.

This test may be used quantitatively if a colorimeter is available, by comparing the depth of colour formed with that produced by a standard nitrate solution of known strength.

8. TOTAL ORGANIC CONTENT

In soils which contain no free carbonates or only a small amount, an approximate idea of the organic matter present in the soil can be obtained by the simple process of burning.

Weigh out 2 or 3 gm. of oven-dried, 1 mm., sieved soil, place it in a weighed crucible and ignite over a large Bunsen burner, or in a small crucible furnace, to bright red heat. Stir occasionally with a steel rod and continue heating for about fifteen minutes. Cool in a desiccator and weigh. The loss represents loss by burning of the organic matter, together with any elemental Carbon which may have been present, and the loss of Carbon dioxide from carbonates. This may be counter-balanced to some extent by a gain from oxidation, but at best the result is only an approximation.

An improvement in the method is to carry out the combustion in a hard glass tube in a current of air freed from Carbon dioxide. The Carbon dioxide produced by the ignited soil is absorbed by Soda lime in a U-tube and weighed. From this Carbon dioxide the weight of Carbon burnt is calculated, and this latter amount is then multiplied by a conventional factor 1·724, on the assumption that soil organic matter contains an average of 58 per cent. of Carbon.

Soils should not be ignited in the laboratory without careful attention to ventilation, as dangerous amounts of Carbon monoxide may be produced if aeration of the ignited sample is restricted.

9. OXIDIZABLE ORGANIC CONTENT

Hydrogen peroxide removes from the soil the portion of the organic matter which is readily oxidizable, that is, the part which is easily available for plant nutrition, and this is to be preferred as a method for humus estimations.

Weigh out about 1 gm. of oven-dried 1 mm. sieved soil into a tall beaker. Add 10 c.c. of water and 10 c.c. of 20 vol. Hydrogen peroxide. Warm on a water bath, stirring occasionally, until bubbling has ceased. Add another 10 c.c. of Hydrogen peroxide and continue heating for a further five minutes, or until all action has stopped. Filter, preferably through an asbestos pad in a Gooch crucible. Wash well with hot water and add the washings to the filtrate. Dry the residue in the crucible at 110° C., cool and weigh. Evaporate the filtrate, or a portion of it, to dryness in a weighed dish. Ignite the residue and weigh. This gives the weight of soluble mineral matter extracted. The weight of organic matter oxidized (O) equals the weight of the original dry soil (W_1), less the weight of the residue in the crucible (W_2) added to the weight of the mineral residue from the filtrate (M), or $O = W_1 - (W_2 + M)$.

Section 2. Physiological Observations

A. TRANSPIRATION

While the conduct of extensive physiological experiments out of doors is usually impossible on account of the apparatus involved, there are certain simple experiments which should be made if facilities are available.

For example the natural rates of transpiration should be compared in various situations using the Cobalt chloride method. For this purpose plants of the same species growing under different conditions, *e.g.*, in sunlight and in shade, may be compared, or, alternatively, a comparison of the rate of transpiration of different plants growing under the same conditions may be made. Another comparison might be made of the leaves of a tree at different heights from the ground. Any or all of these observations are of value and should certainly be made on as extensive a scale as possible. Isolated observations, the result of a single experiment, are, however, of little value, and the average of a number of similar experiments is far preferable to a few scattered observations made under varying conditions.

When dealing with various types of foliage it may become necessary to carry out a large number of observations in a limited time, and if the rate of transpiration is slow it is undesirable to wait until the paper has turned fully pink. Moreover, it is by no means easy to obtain a sharp end point.

The following modification of the standard method, suggested by Dr L. J. Audus, is carried out as follows : Strips of filter paper are dipped into solutions of Aniline Blue of such strengths as to give, when dry, two distinct blue tints. One should be nearly, but not quite, as dark as the blue Cobalt chloride paper, the other should be very pale, almost white. A large stock of this paper should be prepared at one time. As permanence of the standards is naturally desirable, they may alternatively be coloured with best quality water-colours, which is, however, a little more difficult. The object is to obtain two different blue shades corresponding to those through which the Cobalt chloride paper passes as it becomes damp (see p. 318).

Two slips of these papers, 1 in. by $\frac{1}{4}$ in., are stuck across a microscope slide, leaving a gap of about $\frac{1}{4}$ in. between them, so that on one side is a dark slip and on the other a light one. A considerable number of these slides should be prepared for future use.

The blue Cobalt paper is prepared by dipping strips of strong filter paper into a 5 per cent. solution of Cobalt chloride and drying them thoroughly in an oven. The paper may be preserved in stoppered bottles over Calcium chloride.

In making observations, one slide prepared with standard colour strips as above, and one plain slide are placed across a leaf, one on each side, so that the leaf is held between them. The colour strips should not be placed in contact with the leaf. The prepared slide may be either on the upper or the lower surface of the leaf, but the water loss from both sides should always be compared if possible. A piece of Cobalt paper is removed with forceps from the bottle and placed between the leaf and the prepared slide, in such a way as to lie under and between the two blue strips stuck on the reverse side of the slide. The slides are held in position with a pad of " bulldog " clips.

A stop-watch is started when the paper has changed to the darker blue standard and stopped when it reaches the lighter tint. This time gives a measure of the rate of transpiration of the leaf.

The time taken for the upper and lower surfaces to produce the change must also be noted separately. Several observations of leaves in as far as possible the same situation should be made. With a group of students several separate readings may be made at the same time.

Since the times recorded are only relative and only directly comparable by means of an arbitrarily selected blue standard tint, it is desirable as far as possible to make a large stock of standard paper so that observations made over a long period of time may be directly comparable. For this reason the paper stock and the prepared slides must be kept in the dark. The standard tints on the slides must be renewed from time to time if they show any signs of fading, but good water-colour standards are reasonably permanent.

B. ATMOMETRY

Measurements of the evaporating power of the air are necessary checks upon measurements of transpiration. Evaporation is inversely proportional to the humidity of the air, and is usually measured by means of a porous surface, the rate of evaporation of which has been compared with that of a free water surface as a standard. Two types of atmometer or evaporimeter are commonly used, one with a candle of porous porcelain, a method chiefly elaborated by Burton Livingston, the other, the Piché type, using a filter paper.

For an atmometer of the first kind procure a filter candle of the Doulton type with a plane or flanged opening (not a nozzle) and attach it in place of a plant to the side arm of a potometer. Readings may then be made as for transpiration measurements. If the atmometer is to be used in direct sunshine it must be given time to warm up, otherwise the expansion of the water counteracts the loss by evaporation.

To calibrate the instrument, measure the area of the surface of the candle by treating it as a cylinder, capped by a hemisphere. Measure the length and diameter of the cylindrical part. The area equals πdl. The area of the hemisphere is $\frac{\pi d^2}{2}$.

Fill a small parallel-sided dish with distilled water to 1 cm. from the rim. Weigh carefully and place beside the atmometer. Compare, by weighing, the amount of water lost by unit area of the water surface with the amount evaporated per unit area of the candle in the same time. This gives a factor by which the readings of the atmometer must be multiplied to bring them to the water standard.

The Piché evaporimeter consists of a graduated tube sealed at one end and closed at the other by a disc filter paper held in place by a spring. The tube is filled with distilled water and turned upside down. Evaporation takes place from the paper and the amount is read from the graduations on the tube. This instrument must also be calibrated against free water, as above. It is very portable and may be adapted for different circumstances by varying the size of the paper disc, in relation to the bore and volume of the tube.

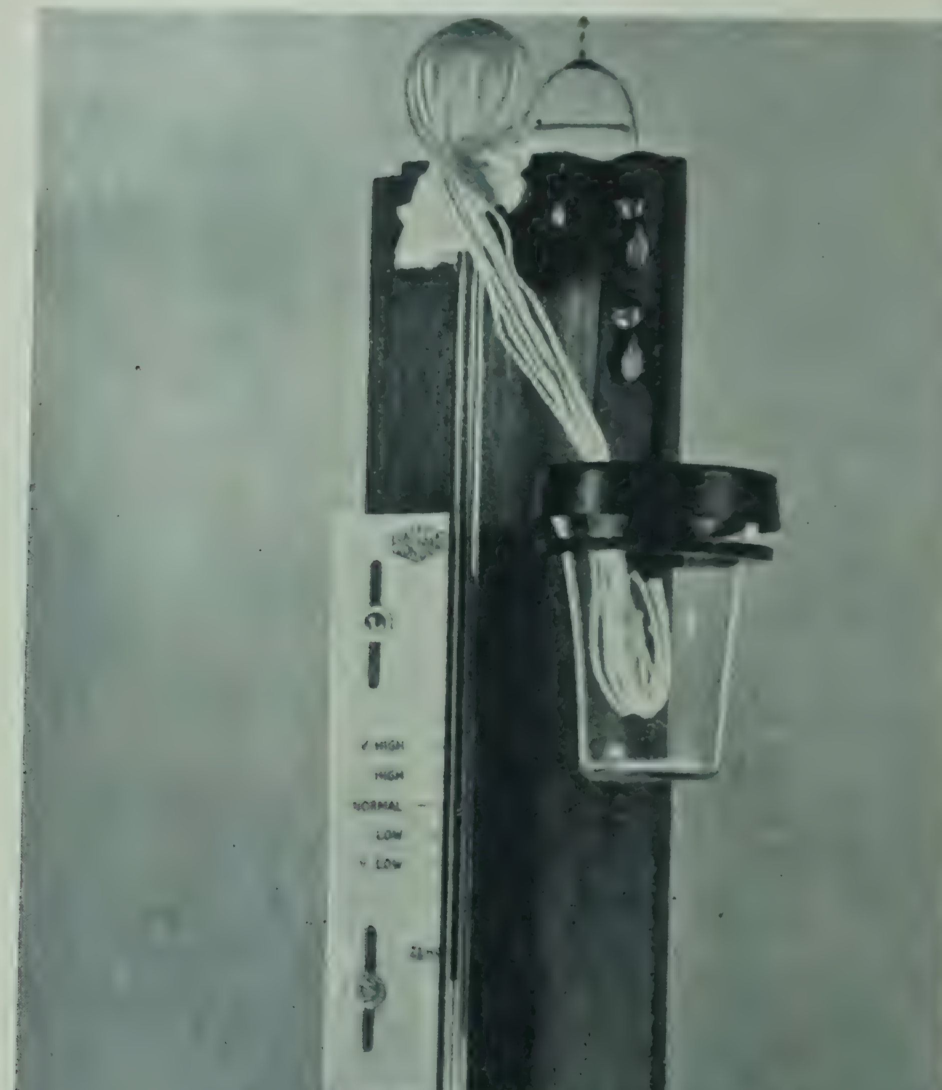
V HIGH
HIGH
NORMAL
LOW
V LOW

FIG. 119.—The Casella atmometer.

Another compact and accurate evaporimeter is made by Messrs Casella and is called the " Rate-of-Drying Meter " (see also Part VII, p. 319, and Fig. 119). It is essentially a differential thermometer, with two bulbs connected by a U-tube manometer containing Mercury. The bulbs are partly filled with ether, over the Mercury, and any difference in the vapour pressure of the ether on the two sides causes movement of the Mercury.

One bulb is swathed in cotton material kept moist by a wick dipping into a water reservoir. The rate of evaporation from the wet bulb controls the difference of temperature between the two bulbs, and hence the rise or fall of the Mercury column which is measured on a scale. The instrument measures the saturation deficit in the air, which is the humidity factor directly controlling evaporation. Like the relative humidity it depends on temperature, but it is a more direct measure of the effect of air on evaporation. For example, air at 3° and at 21° C. may both have a relative humidity of 75, but the rate of evaporation is twice as great at the latter temperature because the saturation deficit is greater.

To calibrate the scale of the Casella instrument, it must be compared side by side with an ordinary wet and dry bulb thermometer, from which the relative humidity can be calculated and then, by reference to tables, the corresponding saturation deficit found. This may involve a little trouble at first, but the measurements thereafter made with this instrument will be perhaps more valuable than those made with the two previous types, and they can, moreover, be made continuously over long periods, which is not possible with the others.

C. ILLUMINATION

1. LEAF MOSAIC

Aesculushippo castanum (Horse Chestnut).—The end portion of a young branch, or a seedling plant, show well what is meant by a **leaf mosaic** (Fig. 120). The longer petioles of the older leaves carry them out beyond the shade cast by the younger leaves above them, at the same time raising them to the same level so that all the leaves near the end of a shoot form a more or less flat-topped corymb.

Further, it will be noticed that the inwardly directed leaf segments are much smaller than the others, minimizing overlapping in the crowded central portion, while the outer segments, which have a free range, are much larger.

In these ways overshadowing is mostly avoided, and the leaves are made to fit together into a ground plan which is characteristic of each species of tree.

Many other species will show similar mosaic arrangements. Notable examples are the Ivy, especially when growing on the ground beneath trees ; the Yew, the leaves of which are produced spirally, but are secondarily bent sideways so as to form two ranks, with the leaves arranged closely side by side, and *Cotoneaster horizontalis*, a wall shrub commonly grown in gardens (see Fig. 121).

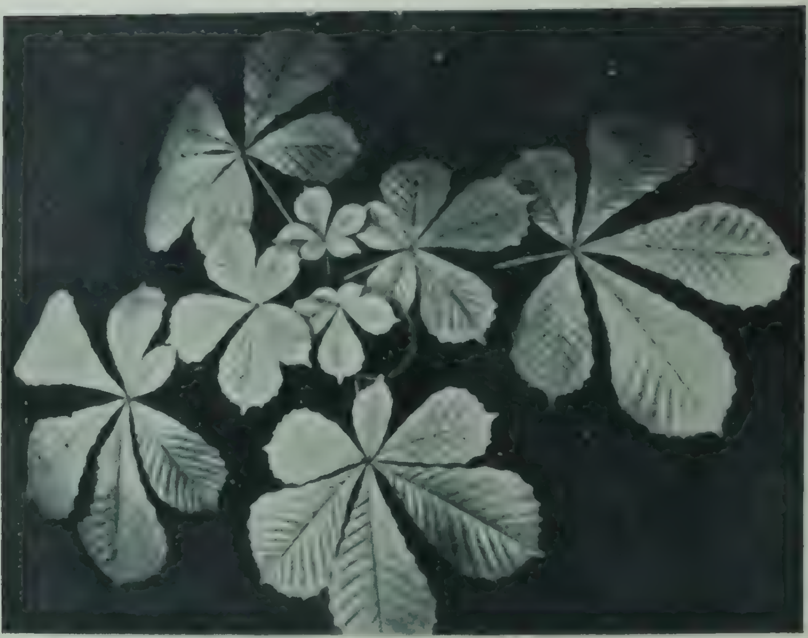

FIG. 120.—*Aesculus hippocastanum.* Young plant showing a leaf mosaic.

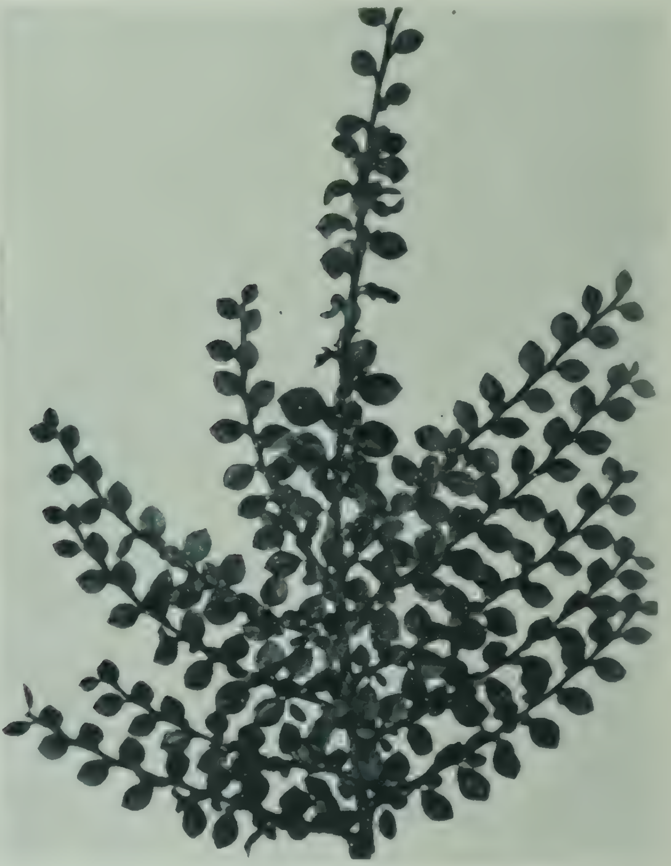

FIG. 121.—*Cotoneaster horizontalis.* A shoot showing the leaf mosaic.

2. ETIOLATION (see also Part VII, Section 4, p. 341)

Seedlings of *Vicia faba* or of *Pisum sativum*, both of which have large reserves of food material in their seeds, make the best subjects for etiolation as they can develop for a considerable time in the dark.

It is important to ensure that the seedlings develop in total darkness, as even very small amounts of light will interfere with the production of the true etiolated form. Similarly, repeated exposures to light, or even one or two minutes daily, while the plants are being examined, will cause marked departures from complete etiolation.

Broad Bean seedlings grown in complete darkness may reach 1 ft. in height. The stem is pure white and has a satiny surface. It ends in a cluster of unexpanded leaves which remain bent over into the characteristic hook form in which they first emerge from the seed. The seedling apex does not straighten out except in the presence of light. These apical leaves are, in fact, those of the primary embryonic plumule, much enlarged but otherwise undeveloped. They have a bright chrome-yellow colour, said to be due to a pigment, **etiolin,** the nature of which is still uncertain. It is probably a carotinoid, perhaps a form of Carotin itself, but some workers have claimed that it is a precursor of Chlorophyll and that it is directly transformed into Chlorophyll on exposure to light.

The stems are quite strong and upright, but the internodes are remarkably long. In one seedling of 6½ in. height there were three internodes, of which the uppermost measured 4 in. At the lower nodes nothing appears but the stipules, and these, though dwarfed in size, are greenish in colour, showing that their Chlorophyll, like that of some cotyledons, can develop in the dark.

Section 3. Herbarium Technique

During work in the field attention should be paid to the accumulation of herbarium specimens of the plants found. This herbarium may serve either to illustrate different ecological associations, or it may be arranged along strictly systematic lines, or both.

In collecting specimens to form such an herbarium attention must be paid to the suitability of individual specimens. In the case of smaller herbs the whole plant should be gathered. It should show not only the typical vegetative characters but should also include, if possible, both the flowers and the fruit. Several specimens of a species should always be collected, especially if there is any marked range of variation in the form or size of the plants. Plants of the same species growing in different ecological habitats will often exhibit marked growth differences. For example, plants growing in a very exposed situation or on sheep-cropped turf will show a great reduction in vegetative growth compared with those living in more protected areas. Notes of these points should be made in the field and added to the data on the specimens when mounted.

Specimens are best collected in a botanical vasculum, which should be of the largest size practicable, so that the plants need not be unnecessarily crushed or bent. A small numbered ticket, such as a price ticket, attached to each specimen and bearing a number corresponding to a number in the field notebook will be found to assist in correlating plants with field notes.

As soon as possible after reaching the laboratory, the specimens should be removed from the vasculum and placed between sheets of drying paper. Proper herbarium drying paper is better than blotting paper because the latter is bleached with chlorine and is liable to discolour the specimens. It also disintegrates easily when damp, and is liable to stick to the specimens. The sheets of drying paper should be of the same size as the herbarium paper used in mounting. The standard size of mounting paper used by the Kew herbarium and by most collectors is 16½ in. by 11 in. The specimens should be carefully arranged on the sheets of drying paper, special care being taken to ensure that the leaves are not folded, and that flowers and fruits are clearly exposed, while at the same time displaying their natural position. Fleshy fruits should not, as a rule, be pressed. Common sense is the best guide what to do. It may often be useful, however, to press one or two flowers separately, sideways, full face and, perhaps, split open, to preserve their details more clearly. If proper drying paper is not available newspaper is a useful substitute and is fairly satisfactory if used in quantity and changed frequently.

When the laying out of specimens is completed the sheets should be placed together in a botanical press. This may conveniently consist of two boards battened across to prevent warping. If the number of sheets being dried is large it may be necessary to intersperse ventilators here and there among the sheets. These consist of frames made of thin laths nailed across one another or of a frame of stout wires soldered together to form a small trellis. Corrugated cardboard may be used instead ; it is not so effective, but is better than nothing. In any case several additional sheets of drying paper should always be interpolated between the sheets carrying specimens, for the purpose of additional absorption and to prevent irregularities in the thickness of one specimen affecting the next. The best weight for a botanical press is half a dozen bricks or their equivalent. Screw-down pressure is to be deprecated, since the pressure is not equally maintained as the plants shrink, but stout webbing straps may be used and are handy for travelling.

The object in pressing and drying plants is to effect the process as rapidly as possible. Perfectly dry paper must be used, and all the sheets must be changed at frequent intervals and the sheets thoroughly dried before being used again. After the plants have been put in the press the paper should be changed within twelve hours, thereafter it need be changed only on alternate days for the first week and subsequently at less frequent intervals. The press should be kept in a dry warm place. New wet plants should not be added to a press of nearly dry plants, but if this is unavoidable a ventilator must be put in between the two lots.

Many collectors like to use artificial means of hastening drying. One popular method is to give each plant, covered with paper, a preliminary pressing with a hot iron before placing it in the press. The results are quite good if overheating is avoided.

A method of rapid drying which has been adopted in the United States, and which presents great advantages, is the use of an infra-red lamp. Rapid drying is always beneficial both for the specimens and for the worker, and this method is very simple.

A 260-watt Mazda infra-red lamp is mounted in a round metal reflector and fixed to a board in an upright lamp socket. A few inches above the lamp is supported a sheet of metal, *i.e.*, zinc, tinplate, sheet steel, etc., to support several sheets of drying paper on which the fresh specimen is laid and covered with a half-filled sand-bag sufficiently slack to flatten out and fit closely over the specimen. The sand-bag acts both as presser and dryer. Flatten it down firmly with the hands and turn on the lamp current (from the house circuit). Drying will be complete in forty minutes to two and a half hours according to the thickness of the specimen but the time may be cut down to ten minutes if the plant is afterwards put into the press to complete the drying.

When perfectly dry the specimens are ready for mounting. Good quality cartridge paper of the standard size should be used, and before the specimens are attached they should be poisoned by immersion or painting with a 1 : 1,000 solution of Mercuric chloride in methylated spirits. Thereafter all specimens should be painted over occasionally with the same poison. This is very necessary to prevent insect attack.

Some collectors prefer to leave the specimens loose on the sheets, but where they are subject to extensive use, as in a public herbarium, they are generally attached by thin strips of transparent adhesive tape or stitched on with needle and cotton. Some collectors use gum, but this is to be deprecated since it prevents the specimen being unmounted and turned over for subsequent critical examination. At the time of mounting the number may be removed and the data from the field notebook transferred to the sheet. These data should include the Latin name, the date when collected, the locality where found and the collector's name. The ecological association of which it is a member and any points of special interest in regard to habitat or variability should be added. Special labels are often enployed, which are printed with the name of the collector or the herbarium of which the sheet forms a part. In tropical or alpine collecting, altitude and other topographical factors may be also included.

All sheets of the same species should be kept together in a white paper folder slightly larger than the size of the sheet and these in turn should be enclosed in coloured folders according to the system of classification selected. If the herbarium is being arranged systematically the coloured folders should have written on the lower left-hand corner the name of the genus and its number, taken from the latest edition of the London Catalogue of British Plants. If the herbarium is arranged ecologically the nature of the association or sub-association should be written on the folder.

The folders, arranged in order, should be stored in a herbarium cupboard specially made to hold them. These cupboards should be fitted with movable shelves arranged every 3 or 4 in. Generally two tiers of shelves are included in a cabinet. The size of the cabinet will depend on the extent of the herbarium and the amount of money available. If possible the cabinet should be made of fire-proof steel and the doors specially constructed with screw fastenings so as to make them almost, if not completely, air-tight. Wooden cabinets should have the doors edged with rubber draught tubing for the same purpose. Naphthalene balls should be included in tin trays in the base of the cabinet. Herbarium specimens preserved in this way will retain their natural appearance indefinitely.

Certain groups of Cryptogams require special treatment. Algae are best preserved by floating them out under water and allowing them to stick to sheets of paper of suitable size, which are raised under the specimens as they lie in the water. After draining off superfluous water the sheets are placed on sheets of drying paper. They must be carefully covered with butter muslin before being placed in the press or the specimens will stick to the drying paper. Small Algae can only be satisfactorily preserved as microscope preparations.

Fleshy Fungi are difficult to dry ; the usual method is to cut a median longitudinal section through the fruiting body and dry this in the press, together with the two halves of the pileus, mounted finally to show both surfaces. Very careful poisoning and drying is necessary for such specimens.

Many smaller Fungi, as well as Lichens and Mosses, can be preserved dry in envelopes mounted on herbarium sheets, care being taken to vary the position of the envelope on the sheets to facilitate storage. More delicate kinds such as Myxomycetes are best preserved dry in small glass-topped boxes or even matchboxes. In any other way they make poor herbarium material.

PART TEN

VARIATION AND HEREDITY

Section 1. Variation

A. FLUCTUATING OR CHANCE VARIATION

1. CONSTRUCTION OF THE CURVE OF CHANCE

The laws of chance may be successfully demonstrated with a dozen pennies. By tossing up the pennies and recording on each occasion the number which fall with their heads uppermost it will be found that there is a tendency towards an equal number falling on their heads and on their tails. This should be repeated about a hundred times to obtain a good average. The labour involved will obviously be lessened if all the members of a class co-operate at the same time, though it must be admitted that certain practical difficulties may be experienced in this. Counters with a spot on one side can, of course, be substituted for pennies.

The results obtained are then plotted on graph paper. Along the base line are set out the numbers 0 to 12, while on the vertical axis are the number of trials. The graph is then constructed showing the number of trials in which each number of heads, from 0 to 12, appeared. When the points so obtained are joined together it will be found that the curve assumes a more or less regular form, rising slowly at first to a maximum at 6 and sinking again to 12.

The regularity and smoothness of the curve will depend upon the number of trials made and the greater the number the smoother its form will be. The curve so produced may be conveniently compared with that obtained for the natural curves of variation obtained by the methods outlined subsequently. The similarity is striking, and emphasises the fact that the fluctuating variation of organisms must be governed by the laws of chance.

2. NATURAL EXAMPLES OF CHANCE VARIATION

Determination of the Normal Curve of Variation in the Length of the Leaves of *Taxus baccata*.

Select a number of branches of Yew and examine them. The mature leaves are all *approximately* the same length. Carefully remove the leaves one at a time and measure them *accurately* with a millimetre rule. If the experiment is carried out by a class the results should be collected and treated as one series, so that if each student measures a hundred leaves the statistics can be based on possibly two thousand measurements. It is important to bear in mind that as large a number of measurements as possible should be made.

Construct a graph, plotting the length of the individual leaves horizontally and the frequency with which each length is recorded vertically. Join the points together and the resulting figure will give a good example of the normal curve of variation. In such a curve it will be seen that while some of the leaves may measure only 18 mm. and others as much as 30 mm., the greatest number will be between 23 and 24 mm. These figures are given from results obtained from one tree. It will not necessarily follow that the same results will be obtained for a different tree, since climatic and other factors make a considerable difference, but in all cases the general shape of the curve will be the same.

Determination of the Curve of Variation in Flower Parts of *Eranthis hiemalis*.

Curves showing the range of variation in the different parts of the flowers can be easily constructed.

Various suggestions may be made, for example, the number of the carpels per flower, the number of nectaries or the number of perianth segments might be selected. A large number of individuals must be examined if a smooth curve is to be obtained. *Eranthis* flowers in early spring, usually in February. Alternative plants may be used if the work is being done at a different time of year. The number of rays on the capitulum of *Bellis perennis* offers convenient material available for the greater part of the year.

In every case the number of individuals should be plotted on the vertical scale and the number of parts along the horizontal scale.

Determination of the Curve of Variation of the Spore Size in *Mucor mucedo* (see p. 84).

Mount a sporangium in water under the microscope and examine the spores. They are oval in shape and either the long or short diameter may be selected for the purpose of statistical treatment. An eyepiece micrometer must be employed and at least a hundred measurements taken.

Before doing this, however, it is advisable to calibrate the scale of the micrometer eyepiece, though, if purely comparative results are all that is required, this may be omitted and the measurements recorded merely in terms of the scale divisions.

The calibration is done with the aid of a micrometer scale, which consists of very fine parallel lines, ruled by machine on a microscope slide, at distances of 0·1 and 0·01 mm. apart, *i.e.*, 100 and 10 μ. Determine by direct observation the length of any group of ten divisions on the eyepiece scale in micra (μ) by comparison with the micrometer scale. From this can be calculated the value in micra of one division of the eyepiece scale. Note that this value will differ according to the objective used and therefore must be calculated for each different objective, including the one to be used in the following work. An average value of one division with a 4 mm. objective is about 3 μ, and with a 2 mm. objective about 1·5 μ.

Replace the micrometer slide by the one containing the spores. Move the slide and, if necessary, rotate the eyepiece so that one of the lines of the eyepiece scale just coincides with the edge of one of the spores, while the spore is lying along the length of the scale. Read off the length, estimating by eye any fraction of a division. Knowing now the factor for converting one division of the scale into micra, record the length of the spore in micra. Repeat the process for 100 spores. The observations will be more accurate if as high a magnification as possible is used. Since the spores are oval, only the length in each case should be measured, although a second series of measurements may be made of spore breadth.

When at least a hundred observations have been made, plot the results on graph paper with the length of the spores arranged horizontally and the frequency vertically. The resulting graph will show the typical curve of normal variation, the peak or "mode" of which shows the most frequent size of the spores of the particular species and strain selected for the experiment. In the case of a truly normal curve this will correspond to the average size.

It will be found that the minimum size of spore length is about 6 μ, while the maximum is about 12 μ. The peak of the curve lying between 9 and 10·5 μ. If spore breadth is used the minimum will be about 3 μ, the maximum about 9·5 μ, while the peak is about 5 to 6·5 μ. As, however, strains of *Mucor mucedo* vary somewhat, and the species are by no means easy to identify, the results may vary considerably from those given above.

Determination of the Curve of Variation in the Size of the Spores of *Equisetum arvense*.

Mount some of the spores of *Equisetum arvense* in water, and after allowing the elaters to separate, measure the diameter of the spores, using the same method as that described previously for *Mucor* (see above). If these results are plotted on a graph in the same way it will be found that a curve is produced which has two maxima. Such a graph is said to be bimodal. The existence of two different types of spores in *Equisetum arvense* can be confirmed by other tests and is of particular interest, for it naturally leads to the suggestion that the species is not completely homosporous, but that a differentiation into two kinds of spores exists, although the fact can only be brought out by careful measurement.

B. CORRELATED VARIATION

Correlation is the term employed to describe the degree of relationship which exists between any two or more quantities. A positive correlation implies a direct relationship of varying degree, and a negative correlation implies an inverse relationship.

Most biological characters are correlated in some degree, depending on the closeness with which they are associated, either morphologically or physiologically, in the organism. A study of correlation is therefore a valuable means of analysis, which often gives information about the constitution of the organism that cannot be obtained by direct observation or experiment. It is important to grasp that correlation may be worked out for purely physiological measurements as well as for morphological characters, though it is often employed in the latter respect, and we will take a morphological example as a simple type.

Students should be encouraged to employ the method and to test correlation for themselves in cases that suggest themselves in the course of study.

The method has sometimes proved of great practical importance, for example in forecasting the probable date of crop ripening where this has been found to be strongly correlated with some previous event in the life history, for example, the date of opening of the first flowers.

The degree of correlation is expressed by a coefficient which varies between $+1{\cdot}0$ and $-1{\cdot}0$. The extremes represent complete (100 per cent.) correlation, either direct or inverse, while the coefficient of 0·0 obviously means that the two quantities are entirely unrelated.

Correlations can be represented graphically by a dot diagram. In this the two quantities are plotted against one another. For example, the correlation between the length and the breadth of leaves may thus be expressed. Make the breadths the ordinates (vertical) of the graph and the lengths the abscissae or horizontal divisions. For every measured length of a leaf place a dot on the graph against the corresponding breadth. If the examples measured are very numerous, as should be the case in this example, it is more usual to group the measures together in groups of ten which fall within certain numerical limits, say, all lengths between 4·0 and 4·5 cm., and plot a dot for the average of the group.

If the two quantities have 100 per cent. *direct* correlation the dots will all fall on a single line sloping upwards, or, in the case of complete *negative* correlation, sloping downwards. If the dots form a circular area it shows zero correlation, while any intermediate oval figure illustrates an intermediate degree approximating more nearly to a straight line the more complete is the correlation.

The calculation of the correlation coefficient is performed as follows :—

Let x and y stand for the two series of measurements and r = the correlation coefficient between them.

Then

$$r = \frac{\Sigma(\delta x \times \delta y)}{\eta(\sigma x \times \sigma y)}$$

where Σ = " the sum of."

δ = the deviation of the measurements in a series from the arithmetic mean of the series.

σ = the standard deviation of a series of measurements.

η = the total number of measurements in a series or of groups in a series.

x and y = the two series of measurements, in this case the lengths and breadths of the leaves.

The deviations (δ) are the differences between each observation in the series and the mean of that series, neglecting sign.

The standard deviation of a series is calculated thus—

$$\sigma = \sqrt{\frac{\Sigma\delta^2}{\eta - 1}}$$

or the square root of: the sum of the deviations squared, divided by the number of measurements less one.

The equation for the correlation coefficient (*r*) may now be expressed in full as follows :—

> " The sum of the products resulting from multiplying the deviation of each *x* measurement by the corresponding *y* measurements, divided by the product obtained by multiplying together the standard deviations of the two series and multiplying the result by the number of measurements in each series."

It will be seen that the arithmetical work involved is considerable, though quite easy to carry out.

Section 2. Mutations

Mutation in *Taxus baccata*, the Common Yew.

In 1780 two female plants of *Taxus baccata* were discovered in the Fermanagh Mountains in Ireland which were of columnar habit, with branches and branchlets quite erect and the leaves standing out all round the twig (Fig. 122). Mr Willis, who found them, transplanted the trees,

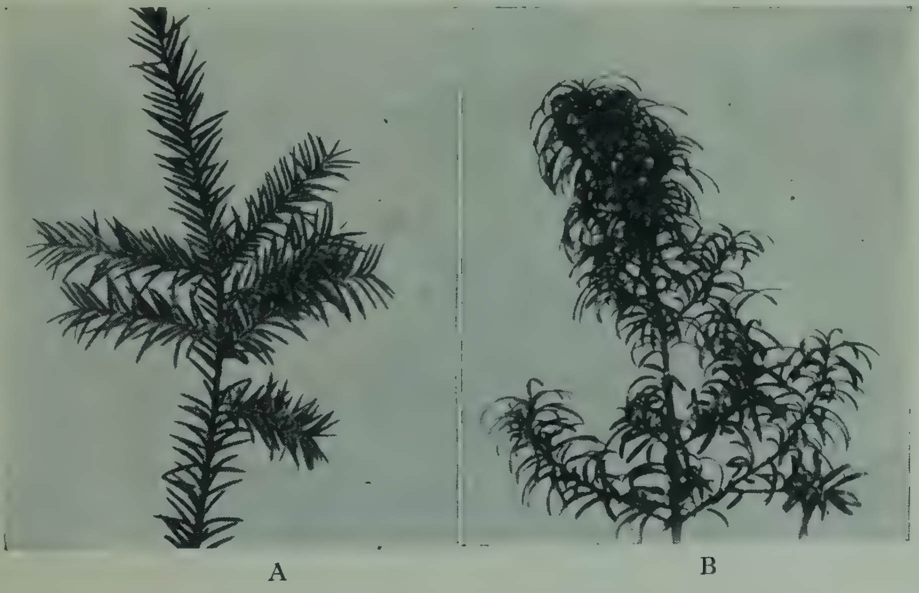

FIG. 122.—*Taxus baccata*. A, normal type. B, mutant type.

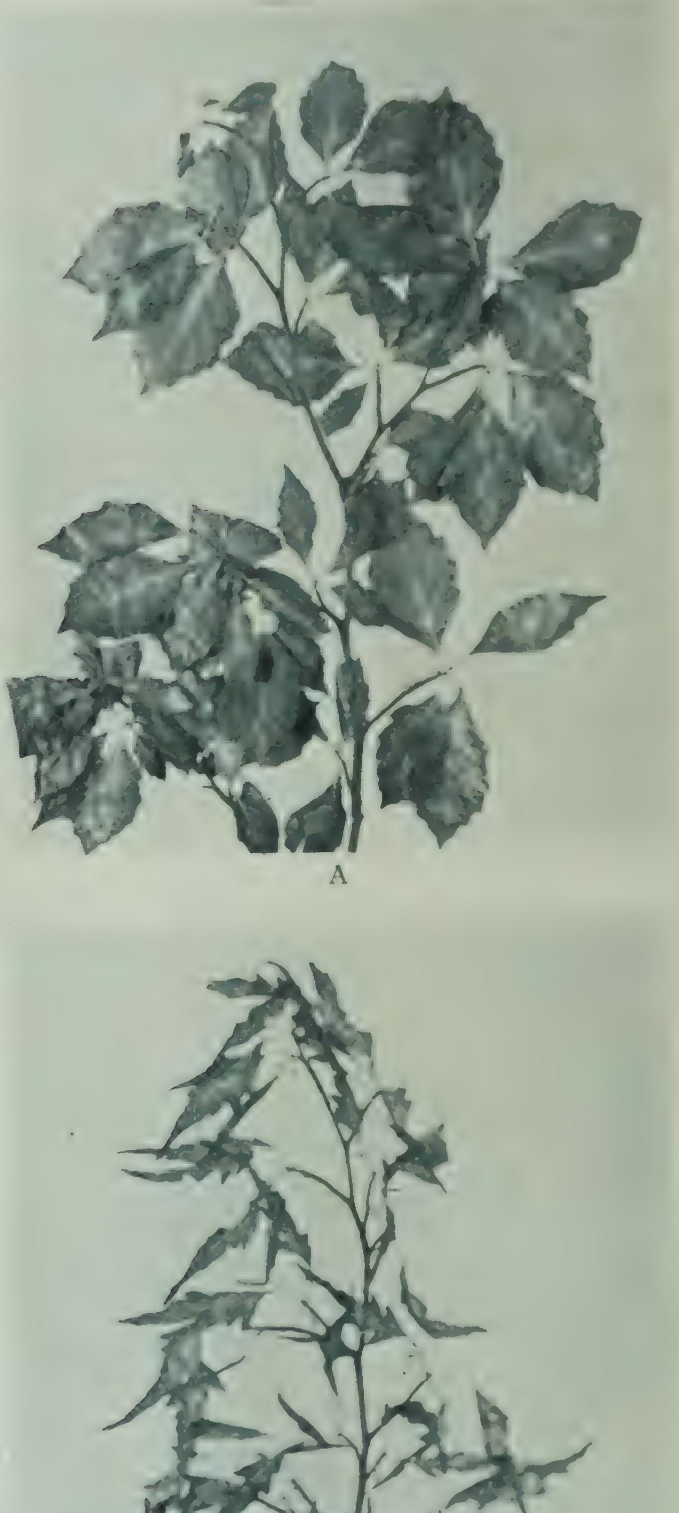

FIG. 123.—*Fagus sylvatica.* A, normal foliage. B, mutant cut-leaved type.

one to his own garden, where it lived till 1865, the other to Florence Court. From this latter tree have been propagated vegetatively all the Irish Yews, though recently several male trees of the same type were found at North Mundham in Sussex. This variety, the Irish Yew, is known botanically as *Taxus baccata* var. *fastigiata* and is considered to be a mutant.

If a specimen of the Irish Yew is available compare it with a shoot of the Common Yew. The differences in growth and arrangement of the leaves will be easily seen, and will serve as a good example of a mutant form in common cultivation which has been propagated solely by vegetative means.

Mutation in *Fagus sylvatica*, the Beech.

Compare a shoot of the normal Common Beech with that of the Copper Beech and the Cut-leaved Beech.

In the Common Beech the leaves are green, oval in shape, 1½ to 2½ in. wide and 2 to 3½ in. long. The margin may be toothed or merely unevenly undulating. In the Copper Beech the leaves are deep purple in colour or even coppery red, while in the Cut-leaved Beech or Fern-leaved Beech the leaves assume various shapes, sometimes long and narrow, 4 in. long by ¼ in. wide ; sometimes deeply and pinnately lobed, the lobes even penetrating to the midrib.

The Copper Beech is known botanically as *Fagus sylvatica* var. *cuprea*, while the Cut-leaved Beech is *F. sylvatica* var. *heterophylla* (Fig. 123). Both these forms have arisen from individual trees which appeared once as a mutation, were recognized as interesting and so were preserved.

Many other common trees, *e.g.*, the Birch and the Lime, exhibit similar mutant forms, which have been propagated and are now widely grown. Various intermediate forms have also appeared, some of which have been preserved and propagated, while others have been allowed to die out. There is always a chance that, by keeping one's eyes open while in the country, a mutant form of some kind may be encountered which might prove of considerable botanical or horticultural interest, and, apart from the chance of its possibilities being recognized, which would be allowed to grow and die without being propagated. Many of these mutant forms come true from seed. This is notably the case with the cultivated Maize plant, which is generally believed to be a mutant form that appeared in ancient Mexico and has been preserved in cultivation for many centuries because of its greater fertility.

Mutation in *Chelidonium majus*, Greater Celandine.

An interesting case of an apparently natural mutation comparable with that in the Cut-leaved Beech is *Chelidonium majus* var. *laciniatum*, the dissected form of the Greater Celandine (Fig. 124). This was first discovered growing at Wimbledon in some quantity about the year 1700. It was brought into garden cultivation as a curiosity, and may sometimes still be found as a garden escape, as it seeds freely and breeds true. In this case the extra division or laciniation of the leaves is shared by the petals, which are cut up into a number of filamentous segments, giving the flower a peculiar, though not particularly attractive appearance.

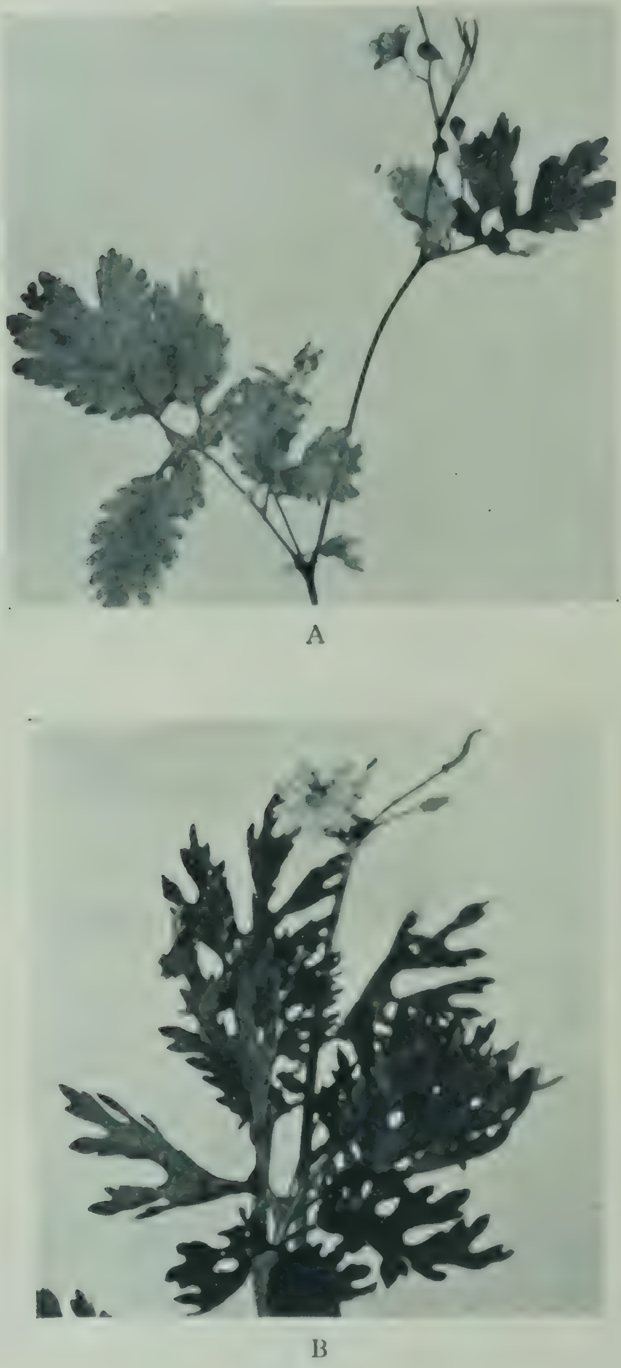

Fig. 124.—*Chelidonium majus*. A, normal type. B, laciniate mutant.

Section 3. Evidences of Evolution

A. ONTOGENETIC EVIDENCE

Acacia melanoxylon or similar species.—Examine living or museum specimens of young and mature plants. In the young stage the leaves have narrow petioles with a central rachis which gives off paired pinnae, each of which produces pinnules in pairs all along its length. Such a leaf is typical of many other members of the Leguminosae, and may be compared with that seen in *Vicia sativa* or other Vetches.

As the plant grows older, in the latter part of the first year or early in the subsequent years, we find that the petioles become progressively flattened, and concurrently the length of the rachis and the number of pinnae are reduced till finally the plant ceases to produce any rachis at all and the whole of the assimilation is taken on by the phyllodic petioles.

Such an example suggests that the ancestral type from which the phyllodic Acacias originated was one which possessed typical leaves without a flattened petiole, and that this condition is still exhibited in the seedling, although gradually given up in after life. This is one of the relatively few cases in the plant world where it may be said that the life history of the individual recapitulates stages in the evolution of the species or that " ontogeny repeats phylogeny."

B. GEOGRAPHICAL EVIDENCE OF EVOLUTION

The geographical distribution of related species forms a very interesting subject of enquiry, and sometimes yields suggestions regarding their possible evolutionary connections. Maps may be constructed on which the areas occupied by each species are marked out and shaded or tinted in a distinctive manner.

Two main types of such maps are : (1) Maps showing the world distribution of the chief genera of a family, or, more simply (2) maps in which different colours show the areas where different numbers of genera occur, which belong to the selected family. Such maps show clearly in many cases what is the geographical centre of distribution of the family, that is, its presumptive place of origin and the routes by which it has spread. This is best shown by families with a tropical or southern tendency, whose general distribution has not been seriously disturbed by the changes of the last Ice Age. The data for such maps can only be drawn from the larger works of reference, such as the " Index Kewensis " or Engler and Prantl, " Die Natürliche Pflanzenfamilien," so that only those who have access to a good library can profit by them. Such works should, however, be available in every library of university rank, and they are also in many of the public libraries, so that opportunities are not so restricted as might appear.

The second type of distribution map deals with the distribution of the species of a single genus and may be restricted to a single geographical area, *e.g.*, the British Isles or Europe. For this purpose a good flora, such as Hooker's "Students' Flora of the British Isles," which gives an outline of the world distribution of each species, is usually sufficient as a source of information, though if more detail is required for Britain, then Druce's "Comital Flora of the British Isles" should be consulted.

Outline maps are easily obtained, and, given the data, the work is easy and the results always interesting, sometimes puzzling and sometimes surprisingly suggestive of the course of evolution, especially when considered in the light of the characteristics of the plants concerned. Although it is scarcely an exercise for a whole class, the study of distribution and the deductions which may be tentatively drawn from this study are attractive employment for a single student or a small group of particular merit.

There are certain publications which may be usefully consulted with regard to this type of work, illustrative of its theoretic value, namely :—

Bews, J. W. : "Some General Principles of Plant Distribution as Illustrated by the South African Flora," *Annals of Botany*, Vol. 35, p. 1. 1921.

Good, D'O. : "The Geography of the Flowering Plants." Longmans, Green & Co. 1947.

Thoday, D. : "The Geographical Distribution and Ecology of Passerina," *Annals of Botany*, Vol. 39, p. 175. 1925.

Willis, J. C. : "Age and Area." Cambridge. 1922.

Section 4. Hybridization and Inheritance

A. EXPERIMENTAL HYBRIDS

Segregation in the Cob of *Zea mais*.

Examine examples of Maize cobs exhibiting Mendelian segregation. The cobs of Maize are specially suitable for demonstrating the segregation of characters. Cobs specially bred for demonstration purposes can be obtained from some laboratory outfitters. Various factors are employed which produce certain easily recognizable characters in the separate fruits. If, for example, a plant whose fruits are coloured purple is crossed with one possessing white fruits, the result in the F1 generation will be the production of fruits in the ratio of three white to one purple in any selected cob. On the other hand, red and white fruits give the opposite result. Count the number of each type of fruit in the cob and calculate the ratio from these figures.

The following common examples may be cited :—

Red fruits to white fruits are in the proportion of	3 : 1
Smooth (starchy) : shrunken (sugary)	3 : 1
Non-coloured : coloured	3 : 1

Similar comparisons may be made with a dihybrid ratio which can be treated by the student in the same way.

The following examples may be cited :—

Yellow starchy fruits to sugary white fruits occur in proportion of 9 : 3 : 3 : 1. A similar ratio occurs with purple starchy to white sugary fruits. Many other hybrid ratios may be found, and the student must work out the ratio from the particular specimen provided. It will be noted that the greater the number of fruits counted the closer will the observed figures approach the ideal ratios.

Breeding Experiments with *Pisum sativum*, Garden Pea.

Where time and opportunity permit, *i.e.*, in a course extending over more than one year, interesting breeding experiments may be profitably conducted by repeating the Mendelian experiments with Garden Peas. Green and yellow Peas are perhaps most easily used, though smooth and wrinkled ones can be obtained equally well. If the flowers of a Pea plant grown from a pure line green Pea be crossed with those of a pure line yellow Pea, an F1 generation of hybrid seeds will be obtained. This crossing is best carried out by removing the stamens from one flower which is still unopened, and, when it is mature dusting the stigma with a camel-hair brush charged with pollen taken from the ripe anthers of a flower of the other race. To obviate the risk of interference by insects the selected flowers should be enclosed in paper bags, such as are used for sweets, which are secured round the stalk by string or rubber bands until the fruit has set.

After the seed collected from these flowers has been carefully dried it should be sown. As the flowers of the next generation appear, each should be enclosed in a bag and left. In the case of the Pea this is not absolutely essential as the flowers are habitually self-pollinated, but it is necessary in most other cases and is generally a valuable precaution.

When the pods are ripe they are collected and opened. A record is made of the number of green and yellow seeds in each pod. From the results the proportion of green and yellow seeds can be found, and these should work out in the proportion of approximately three green seeds to every yellow one, provided that the original green and yellow seeds were from pure lines.

Some of these seeds may be sown, the flowers then self-pollinated and the results recorded for each plant. In this way the pure green and the heterozygous green Peas of the F1 generation can be separated.

Obviously even under greenhouse conditions such an experiment will take a long time, and where such work is impossible quite interesting dried examples can be obtained from the General Biological Supply House Inc., of East 69th Place, Chicago, showing seeds obtained as a result of such Mendelian experiments.

As alternatives to the Pea, varieties of garden Snapdragons could be used. For example, the crossing of a white variety with a coloured one would provide good material. In such an example the stamens of, say, the white flowers should be cut off at an early stage, and later, when the stigmas have separated, a ripe stamen from a coloured flower is rubbed on to them. The seeds so obtained should be sown and allowed to flower.

If time permits these should be self-pollinated and sown again the following year, when the Mendelian segregation of the two colours should appear.

Similar experiments with white and purple flowered Sweet Peas will in many cases yield a pink heterozygous form in addition to the segregated whites and purples.

B. NATURAL HYBRIDS

Natural Hybridization in the Genus *Geum*.

Where plants of *Geum rivale* and *Geum urbanum* occur commonly together a natural hybrid known as *Geum intermedium* frequently appears. If fresh material or herbarium specimens of the three are available a comparison may be made with the aid of a dissecting microscope.

The most important features for study are the receptacle, calyx and carpel. In *G. rivale* the calyx is cup-shaped and only the tips of the sepals are reflexed. The receptacle is long and bears carpels with a feathery stigma. In *G. urbanum* the calyx is completely reflexed and the receptacle short, bearing carpels with a smooth hooked stigma. In the hybrid *G. intermedium* it will be seen that at the same age the calyx is made up of five sepals which stand out horizontally. The receptacle is short and the style smooth like that of *G. urbanum*. Thus the hybrid possesses certain intermediate features, while in other respects it resembles one only of the two parents.

Natural hybrids are common among the Willows, the Mints, the Willowherbs and the Violets (see Fig. 125), to mention only a few examples. Every opportunity should be taken to observe and study any hybrids found in the field, which may be identified from a Flora or in difficult cases by reference to a museum specialist.

C. VARIATION AND HYBRIDIZATION WITHIN NATURAL SPECIES

Many of the recognized species defined by the older authors, such as Linnaeus, especially those which were said to be " very variable " or " polymorphic," have proved on close study to be made up of a number of " sub-species," distinguished from each other by small morphological characters or by differences of habit, such as flowering season, which are relatively constant and can be proved to be hereditary. Such species are therefore often called " aggregate species " or Linneons, and their sub-divisions " micro-species," " elementary species " or Jordanons, after Alexis Jordan, who, in 1864, first drew attention to the very numerous constant forms which make up the species *Draba* (*Erophila*) *verna*, the common Whitlow Grass.

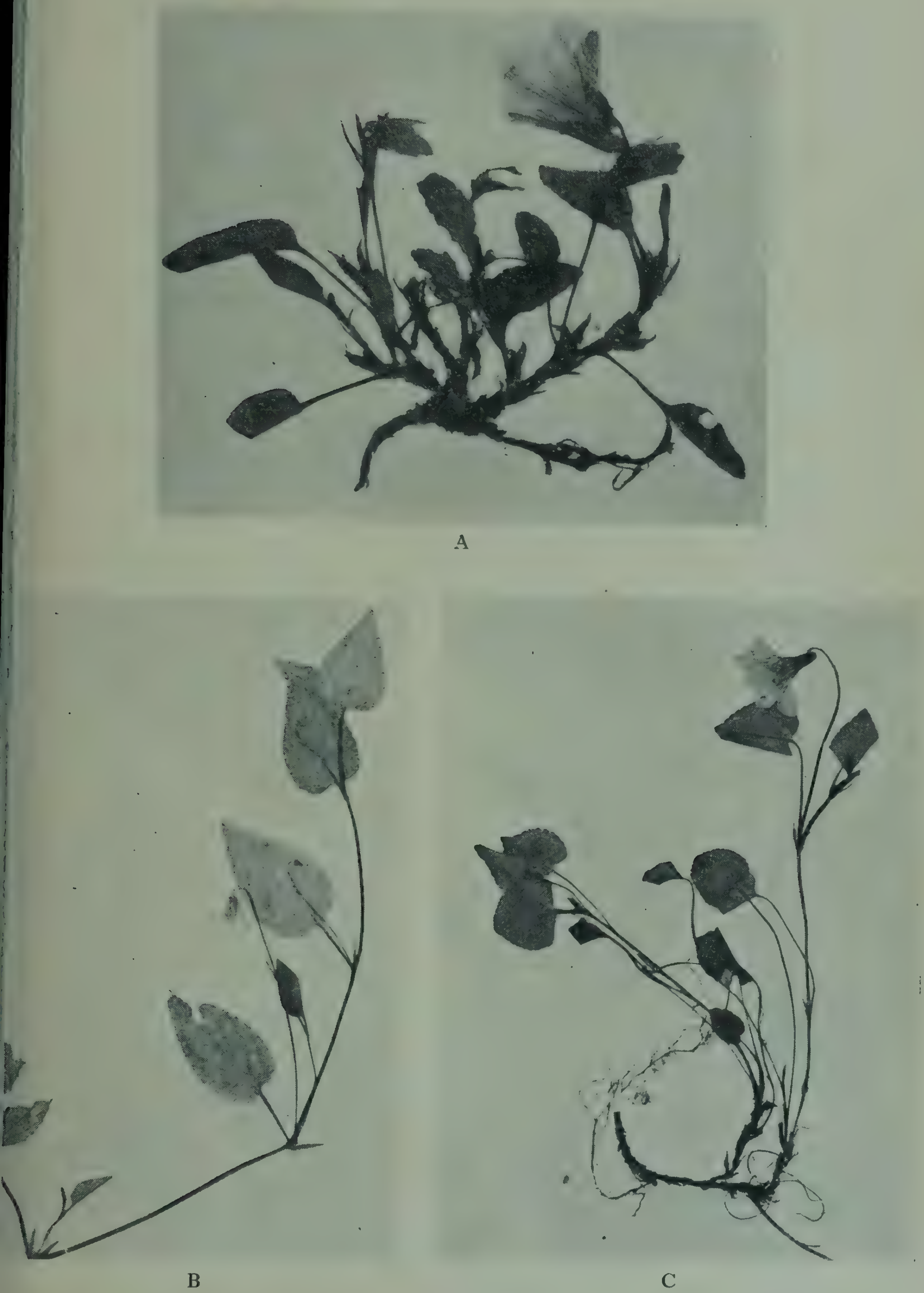

FIG. 125.—*Viola.* Natural hybrid between *V. lactea* and *V. riviniana.* A, *Viola riviniana.* B, *Viola lactea.* C, Natural hybrid of *V. lactea* and *riviniana.*

These facts should be borne in mind in estimating the evolutionary value of the small "variations" utilized by Darwin as material for his theory.

Several common plants are available for the study of such micro-species. The classic type, *Erophila verna*, is a very small plant and its forms are mostly local, so that all the plants in a small district may belong to the same form. For this reason we have selected the following as practical examples :—

Capsella bursa-pastoris (L.) Medic.—In *Capsella* the leaves of the basal rosette display a remarkable variety of forms. One extreme type was called by Lotsy var. *taraxacifolia*, with leaves deeply divided into sharply pointed pinnate segments resembling, as the name implies, the leaves of some types of Dandelion, particularly *Taraxacum erythrospermum*.

The other extreme is represented by var. *integrifolia*, with broad, entire leaves like those of a Forget-me-not. These leaf types, however, show true variability, they are not strictly hereditary and do not by themselves distinguish constant taxonomic units, *i.e.*, they are varieties in the older sense of the word.

The fruits are, however, also of very various forms and these appear to be distinct, constant and hereditary. Taken in conjunction with the leaf form they serve to separate a number of micro-species which are real units, sub-species of the aggregate *C. bursa-pastoris*.

Dr Almquist of Stockholm has cultivated and described about two hundred of these sub-species. As the flowers are mostly self-pollinated these forms do not often cross-breed and are presumably homozygous and show only narrowly fluctuating variations within their own limits. Eighteen such types have been found in Britain.

Plantago coronopus Linn.—This species presents a rather different state of affairs. It is commonly to be found in turf near the sea and presents, in most localities, a baffling medley of leaf forms so diverse that they scarcely seem to belong even to the same aggregate species. These vary between var. *pygmaea* Lange, with minute entire leaves, and var. *latifolia* de Candolle, in which the leaf is upwards of 4 in. long, nearly ½ in. broad and has three to five parallel veins and doubly pinnate segments. Here the majority of the forms probably represents hybrids between a small number of basic types. In contrast to *Capsella* the flowers, being anemophilous, are freely cross-pollinated, so that the various sub-species are more or less obscured by hybridization.

It is an interesting study to arrange a collection of mature leaves according to type (see Fig. 126), whereby some idea may be gained of the probable number of elementary species involved and of their inter-crossing.

Taraxacum officinale Weber.—A very large number of micro-species have been segregated from this species by various authors, notably by Dahlstedt between the years 1901 and 1912, based upon both leaf and flower characters. The variability of the leaves is immense, as in the other two cases cited above, and constant types cannot be distinguished on the leaf form alone, especially as a good deal of difference exists between younger and older leaves, even on the same individual. As *Taraxacum* is habitually parthenogenetic, it is evident that each of the elementary species is genetically isolated and will therefore tend to persist indefinitely.

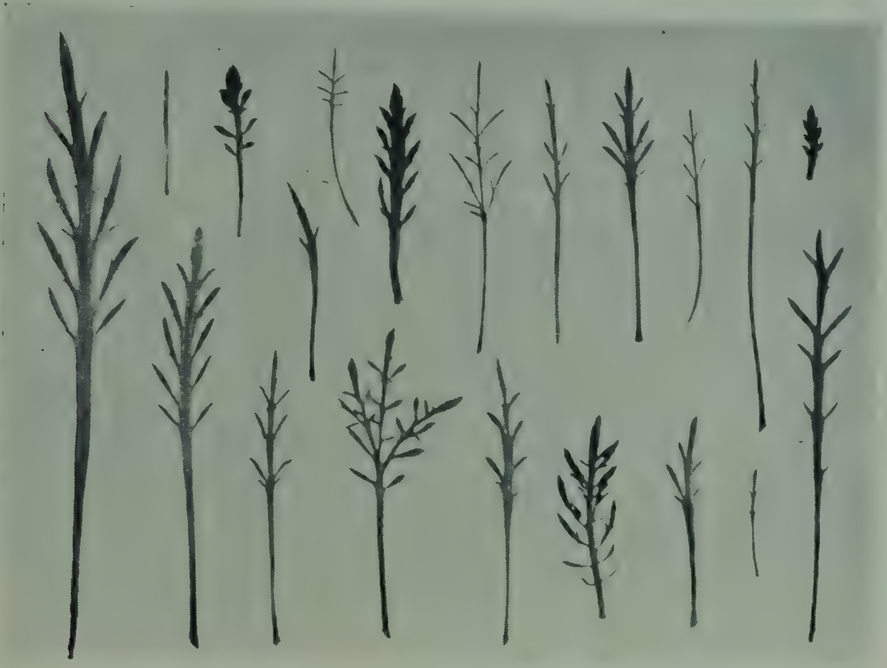

FIG. 126.—*Plantago coronopus*. Variation in leaf form.

It will be seen that, in view of such examples as the above, the old idea of polymorphic species appears to break down, on fuller knowledge, into one of aggregate species, inside which each elementary species is distinct, although usually showing its own narrow range of fluctuating variability, dependent on the environment and on its stage of development.

D. GRAFT HYBRIDS

Laburnum adami.—This graft hybrid originated in 1825 in the garden of a nurseryman named Adam, near Paris. It was artificially produced by grafting a scion of *Cytisus purpureus*, the Purple Broom, on a stock of *Laburnum anagyroides*, the common Laburnum. In this particular instance the scion appeared to have perished, but at its side an adventitious shoot subsequently arose which produced foliage intermediate in size, and flowers intermediate in colour, between *Cytisus purpureus* and *Laburnum anagyroides* (Fig. 127). This graft hybrid has only once appeared, for the hybrid does not reproduce itself from seed, and all existing plants have been derived by cuttings from the original plant. It is frequently planted as an ornamental tree and is obtainable from most nurserymen. In old specimens segregation takes place, so that all three types may be found on one tree.

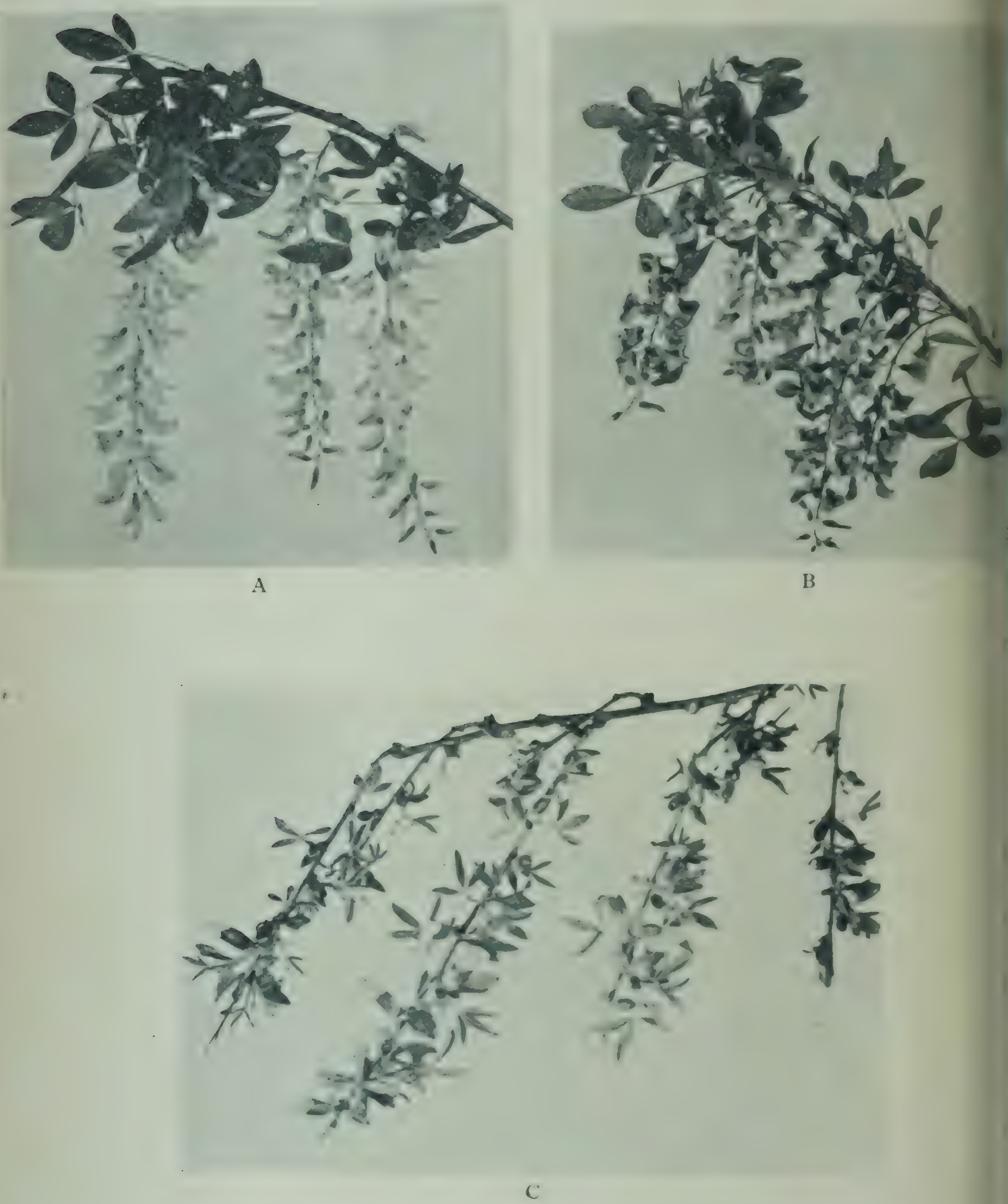

FIG. 127.—*Laburnum adami*. A graft hybrid of *Laburnum anagyroides* and *Cytisus purpureus*. A, *Laburnum anagyroides*. B, *Laburnum adami*. C, *Cytisus purpureus*.

Compare specimens of the hybrid and of the two parents. The leaves have the shape of the Laburnum but lack the hairs on the lower surface. The inflorescence is like Laburnum, but the flowers are pinkish purple and set few fertile seeds. Those which are formed invariably produce pure Laburnum offspring.

Cut a section through a branch of each of the three examples and stain in Aniline chloride and mount in Glycerine.

In the section of *Laburnum anagyroides* the cork layer arises just below the epidermis, leaving the epidermis itself free. In the case of *Cytisus purpureus* the cork layer arises actually from the cells of the epidermis. In *Laburnum adami* the cork arises from both layers, showing the anatomical combination which has occurred in the formation of the hybrid.

Various other graft hybrids are known, of which the hybrid between the Hawthorn, *Crataegus monogyna* and the Medlar, *Mespilus germanica*, may be cited. If an opportunity occurs compare the branches of the two parents and of the graft hybrid known as *Crataego-mespilus*, which has foliage resembling that of the Medlar, but fruits which are intermediate in size and colouring.

Artificially produced graft hybrids can also be obtained by the union of Potato and Tomato material. Very interesting hybrids have been produced in this way, and students might attempt to copy the work of Crane and Lawrence, if greenhouse facilities exist, though it must be pointed out that considerable skill is necessary to make successful unions, and that luck plays no small part in the results.

APPENDIX

Receipts for some Common Stains and Reagents used in this Book

It is not proposed in this appendix to give anything more than the receipts of the reagents most commonly employed in the botanical laboratory. Many and varied are the receipts and methods employed both by teachers and research workers, and the requirements of any two laboratories are not likely to be the same. In order, however, to help the teacher, and more particularly the independent worker, the receipts for the solutions mentioned in the text have been included, except where the directions for making them up are given in the description of their use.

It is assumed that the laboratory will possess the usual dilutions of acids and alkalis, together with the more common test reagents and no attempt has been made to include instructions for such as these.

It cannot be too strongly emphasised that students should be encouraged to experiment with different fixatives, stains and tests, so far as the facilities of the laboratory will allow. There is no better way than this of gaining an insight into microscopic technique.

Those who require fuller details of the preparation of reagents for botanical laboratories are referred to the account given by the present authors in their book, "Plant Science Formulae, a Reference Book for Plant Science Laboratories," Macmillan, 1941.

The supply of Alcohol for microscopy is attended with certain difficulties. Ordinary Methylated Spirit does not make clear mixtures with water and cannot be used. The Toilet Quality, which costs slightly more, is, however, satisfactory. This can be obtained in various strengths, of which that of 74 over proof is equivalent to absolute Alcohol and may be generally used in its stead. Pure absolute Alcohol, as ordinarily sold, is extremely expensive but a permit for the purchase of tax-free absolute Alcohol may be obtainable on application to the local Excise Office. The purchase of Toilet Quality spirit also requires an Excise permit.

The use of Ethyl Alcohol may be avoided altogether, if necessary, by using Isopropyl Alcohol, which is much cheaper than absolute Ethyl Alcohol bought in the ordinary way.

Section 1. Microscopical Reagents

A. FIXATIVES

1. **Form-Alcohol** (Chicago Formula).

Alcohol 70 per cent. . . .	100 c.c.
Formalin	6 c.c.

Material may be fixed and stored in this fluid indefinitely.

2. **Universal Fixative** (Rawlin's Formula).

Alcohol 50 per cent. . . .	100 c.c.
Formalin	6·5 c.c.
Glacial Acetic acid . . .	2·5 c.c.

3. Chromic Acid Fixative.

Chromic acid	10 gm.
Glacial Acetic acid	10 gm.
Water	1,000 c.c.

Fix for twenty-four hours and wash in water. Various other strengths may be made up. Weaker solutions are needed for more delicate material.

4. N.K.L. Fixative (Navaschin-Karpechenko-Lenglet Formula).

Solution A—		*Solution B—*	
Chromic acid	1 gm.	Formalin	40 c.c.
Acetic acid	10 c.c.	Water	35 c.c.
Water	65 c.c.		

Use equal parts; it is frequently better to dilute to half strength. Material may be left fixing for some time.

5. Bouin's Fluid.

Picric acid (1·5 per cent. aq.)	75 c.c.
Formalin	25 c.c.
Glacial acetic acid	5 c.c.

Fix for twenty-four hours. Wash in water and grade up through Alcohol; store in 75 per cent. Alcohol.

6. La Cour Fixatives.

"2.BD"

Chromic acid 1 per cent.	100 c.c.
Potassium dichromate 1 per cent.	100 c.c.
Acetic acid 5 per cent.	30 c.c.
Osmic acid 2 per cent.	30 c.c.
Saponin	0·1 gm.

"2.BE"

Chromic acid 1 per cent.	90 c.c.
Acetic acid 5 per cent.	10 c.c.
Osmic acid 2 per cent.	15 c.c.
Potassium dichromate	1 gm.
Saponin	0·05 gm.

The material should be well washed and bleached with Hydrogen peroxide (1 part to 4 parts of 80 per cent. Alcohol).

7. Flemming's Solution.

Chromic acid 1 per cent.	75 c.c.
Glacial acetic acid	5 c.c.
Osmic acid 2 per cent.	20 c.c.

Fix for twenty-four hours, wash and grade through Alcohol. Bleach with Hydrogen peroxide. May also be used in a weaker concentration for more delicate material.

B. MACERATING FLUIDS

1. Schulze's Macerating Fluid.

Potassium chlorate	1 gm.
Nitric acid	50 c.c.

The material should be heated in this mixture for a short while. It should then be washed and mounted in Glycerine.

2. Ammonium Oxalate Macerating Fluid.

Fresh material is treated for twenty-four hours in a solution of Acid Alcohol, prepared by adding 30 c.c. of Hydrochloric acid to 100 c.c. of 95 per cent. Alcohol. It is then washed in water and transferred to 0·5 per cent. Ammonium oxalate and boiled. The tissues should be mounted in a drop of the solution and tapped gently to separate the cells.

3. Harlow's Medium.

First place the material in strong Chlorine Water for three hours, then wash. Place in 3 per cent. Sodium sulphite solution and warm for fifteen minutes ; wash. Repeat till the material falls apart.

C. STAINS

1. Ehrlich's Haematoxylin.

Haematoxylin	1 gm.
Glacial acetic acid	5 c.c.
Glycerine	50 c.c.
Absolute alcohol	50 c.c.
Distilled water	50 c.c.
Potassium aluminium sulphate in excess.	

Keep in a dark place till the colour becomes dark red. It will keep indefinitely if well stoppered and is therefore an improvement on the Delafield's formula sometimes employed.

2. Heidenhain's Haematoxylin.

Solution A—2 per cent. solution of Ferric ammonium alum in water.
Solution B—0·5 per cent. solution of Haematoxylin in distilled water.

The Haematoxylin crystals will take about ten days to dissolve in the water. The stain reaches its greatest efficiency in about six weeks. It deteriorates in about three months. A stain made by dissolving the Haematoxylin in a little strong Alcohol and then diluting the Alcohol is not as good, but is usable.

3. Safranine.

Safranine	1 gm.
Alcohol 95 per cent.	50 c.c.
Water	50 c.c.

4. Spirit (Aniline) Blue.

Spirit Blue	1 gm.
Alcohol 95 per cent.	100 c.c.

5. Gentian Violet (Crystal Violet).

Gentian Violet	1 gm.
Water	100 c.c.

May also be made up in 95 per cent. Alcohol.

6. Bismarck Brown (Vesuvine).

Bismarck Brown	2 gm.
Alcohol 70 per cent.	100 c.c.

7. Eosine.

Eosine	1 gm.
Water or 70 per cent. alcohol	100 c.c.

Various forms of Eosine are now on the market: some more soluble in water, others more soluble in Alcohol.

8. Erythrosine.

Erythrosine	1 gm.
Absolute Alcohol	5 c.c.
Clove oil	95 c.c.

Dissolve the stain in the absolute Alcohol and then add the Clove oil.

9. Light Green.

Light green	1 gm.
Absolute Alcohol	25 c.c.
Clove oil	75 c.c.

Dissolve the stain in the Alcohol and then add the Clove oil.

10. Basic Magenta (Fuchsine).

Basic magenta	1 gm.
Alcohol 95 per cent.	100 c.c.
Water	100 c.c.

11. Carbol-Fuchsine (Ziehl-Neelsen).

Basic Magenta (Fuchsine)	0·3 gm.
Absolute Alcohol	10 c.c.
Phenol	10 c.c.
Water	200 c.c.

Dissolve the Fuchsine in the absolute Alcohol and then add the Phenol, dissolved in the water. The solution keeps well.

12. Methyl Green (Iodine Green).

Methyl green	1 gm.
Alcohol 50 per cent.	100 c.c.

13. Methyl Green, Acetic.

Methyl green	1 gm.
Glacial acetic acid	1 c.c.
Water	100 c.c.

14. Cotton Blue (Soluble Blue) in Lacto-Phenol.

Soluble blue	1 gm.
Lacto-phenol (see p. 458)	100 c.c.

Fresh material may be mounted in this fluid.

15. Picro-Aniline Blue.

Picric acid (8 per cent. in 95 per cent. Alcohol)	78 c.c.
Spirit blue (1·5 per cent. in 95 per cent. Alcohol)	22 c.c.

Stain for two minutes, wash in absolute Alcohol and dehydrate as rapidly as possible.

16. Picro-Nigrosine.

To 100 c.c. of aqueous 1·5 per cent. Picric acid add 1 gm. of Nigrosine and filter. Used as a combined stain and fixative with fresh material, especially of Algae.

17. Polychrome Blue.

Polychrome blue	0·5 gm.
Phenol	1·0 gm.
Alcohol 95 per cent.	10 c.c.
Water	100 c.c.

Dissolve the Phenol in the Alcohol and grind up with the stain in a mortar. Add two-thirds of the water and stir actively. Pour into a bottle and wash out the mortar with the rest of the water.

D. MOUNTING MEDIA

1. Glycerine Jelly.

Gelatine	1 part.
Glycerine	7 parts.
Water	6 parts.

Warm the Gelatine in the water for about two hours, then add the Glycerine. Warm and stir for fifteen minutes and add 1 per cent. of Phenol as a preservative. The mixture may be cleared, if necessary, by filtering while warm, through paper pulp or thick filter paper in a Buchner funnel.

2. Lacto-Phenol.

Phenol	100 gm.
Glycerine	100 c.c.
Lactic acid	100 c.c.
Water	100 c.c.

Dissolve the Phenol in the water without heat to prevent oxidation. Then add the Glycerine and Lactic acid.

3. Mayer's Albumen Fixative for Sections.

White of egg	50 c.c.
Glycerine	50 c.c.
Sodium salicylate	1 gm.

Separate the white from the yolk of the egg, dissolve the Sodium salicylate in a very little water and add the other ingredients. The whole is well mixed and then filtered.

Section 2. Chemical Reagents

A. MICROCHEMICAL TEST REAGENTS

1. Iodine Solution (Gram's).

Iodine	1 gm.
Potassium iodide	2 gm.
Water	300 c.c.

First dissolve the Potassium iodide in the water and then add the Iodine.

2. Chloriodide of zinc.

Solution A—		*Solution B—*	
Zinc chloride	20 gm.	Potassium iodide	1·0 gm.
Water	8·5 c.c.	Iodine	0·5 gm.
		Water	20 c.c.

Add Solution B drop by drop to the first solution until a precipitate of Iodine forms, which does not disappear on shaking. This takes about 1·5 c.c.

3. Corallin Soda.

Make a saturated solution of Corallin in 4 per cent. aqueous Sodium carbonate, to which a little Camphor has been added as a preservative. Better used fresh.

4. Ammoniacal Copper Hydroxide (Schweitzer's Reagent).

To a solution of Copper sulphate in water add excess of strong Ammonium hydroxide until the precipitate formed is just redissolved. It must be freshly prepared for use.

5. Aniline Chloride.

A saturated solution is made in distilled water, filtered and acidified with a few drops of Hydrochloric acid. It should give a distinct acid reaction. The solution may also be made up in Alcohol and diluted with water.

6. Phloroglucin.

Dissolve the Phloroglucin in 95 per cent. Alcohol and gradually add strong Hydrochloric acid until it begins to precipitate. The solution is then ready for use.

7. Schiff's Reagent.

Basic Magenta	0·025 gm.
Water	100 c.c.

When dissolved add 20 drops of concentrated Sulphuric acid. Titrate with 1 per cent. Sodium bisulphite until no more colour is lost. With most samples of Magenta there will be some residual colour, which is yellowish.

8. Sudan III.

Saturated solution of the dye in 70 per cent. Alcohol. Warm to dissolve.

9. Alkannin Reagent.

Make a saturated solution in absolute Alcohol and then dilute with 95 per cent. Alcohol. It is best to use the dry root of *Alkanna* for making solutions. It may be obtained through a pharmacy.

10. Ruthenium Red.

Ruthenium red is Ruthenium sesquioxide. Use a 1 : 5,000 solution in water; keep in the dark. Sections should be left for twenty-four hours in acid Alcohol (Hydrochloric acid 1 ; Alcohol 3) and then for some hours in dilute Ammonium hydroxide, followed by the stain.

11. Orcin Solution.

Orcin	0·5 gm.
Alcohol 90 per cent.	100 c.c.

Sections are treated in this and then warmed with strong Hydrochloric acid.

12. Fehling's Solution.

Solution A—

Copper sulphate	34·66 gm.
Water to .	500 c.c.

Solution B—

Potassium sodium tartrate	173 gm.
Sodium hydroxide .	50 gm.
Water to . . .	500 c.c.

Use in equal quantities. The addition of a drop of Methylene Blue to the solution being titrated greatly sharpens the end point when the test is used in quantitative work. The blue colour is discharged after all the Copper sulphate has been reduced.

13. Millon's Reagent.

Mercury	30 c.c.
Nitric acid	570 c.c.
Water	1,200 c.c.

Dissolve the Mercury in the Nitric acid (preferably in a fume cupboard) and then add the water.

14. Eau de Javelle.

Add 50 gm. of chloride of lime and 100 gm. of Potassium carbonate or Sodium carbonate to 1 litre of water. Shake thoroughly. Need not be filtered if allowed to settle before use.

B. INDICATORS

Universal Indicator Solution.

Tropaeolin	70 mg.
Methyl orange	100 mg.
Neutral red	80 mg.
Bromothymol blue . . .	400 mg.
Naphtholphthalein . . .	500 mg.
Cresolphthalein	400 mg.
Phenolphthalein	500 mg.
Alizarin yellow R. . . .	150 mg.

Dissolve the above dyes together in 200 ml. of 70 per cent. Alcohol and use 1 drop in 10 c.c. of test fluid.

Colour Range given by the above Indicator

*p*H.	Colour.	*p*H.	Colour.
2·0	Orange-red	8·0	Green.
3·0	Red-orange	8·5	Blue-green.
4·0	Orange	9·0	Green-blue.
5·0	Yellow-orange	9·5	Violet-blue to blue-violet.
6·0	Orange-yellow	11·0	Violet to violet-red.
6·5	Yellow	12·0	Violet-red.
7·0	Green-yellow		

C. WATER CULTURE SOLUTIONS

Normal Complete Solution (Sachs).

Calcium sulphate	0·25 gm.
Calcium phosphate	0·25 gm.
Magnesium sulphate	0·25 gm.
Sodium chloride	0·08 gm.
Potassium nitrate	0·70 gm.
Ferric chloride	0·005 gm.
Water	1,000 c.c.

(*a*) **Solution minus Calcium.**

For Calcium sulphate use Potassium sulphate 0·20 gm.
For Calcium phosphate use Sodium phosphate 0·71 gm.

(*b*) **Solution minus Iron.**

Omit Ferric chloride.

(*c*) **Solution minus Nitrogen.**

For Potassium nitrate use Potassium chloride 0·52 gm.

(*d*) **Solution minus Phosphorus.**

For Calcium phosphate use Calcium nitrate 0·16 gm.

(*e*) **Solution minus Sulphur.**

For Calcium sulphate use Calcium chloride 0·16 gm.
For Magnesium sulphate use Magnesium chloride 0·21 gm.

(*f*) **Solution minus Magnesium.**

For Magnesium sulphate use Potassium sulphate 0·17 gm.

(*g*) **Solution minus Potassium.**

For Potassium nitrate use Sodium nitrate 0·59 gm.

D. AGAR MEDIA

1. Corn Meal Agar.

Corn (Maize) Meal	50 gm.
Water	1,000 c.c.

Warm at a temperature of 58° to 60° C. for one hour. Filter and add 1·5 per cent. of Agar. Steam for ninety minutes. Filter and tube. Autoclave for twenty minutes at 120° C. Suitable for growing Phycomycetes.

2. Malt Agar.

Malt extract	10 gm.
Agar	7·5 gm.
Water	500 c.c.

Dissolve the Agar in the water and filter through paper pulp. Add the malt extract dissolved in a little hot water. Autoclave for twenty minutes at 120° C. A good general agar for Fungi.

3. Potato Agar.

Grate 500 gm. of clean Potatoes into water and boil gently for about half an hour. The extract is allowed to cool and settle as much as possible and the supernatant water poured off and made up to 1,000 c.c. with water. Place in a flask with 25 gm. of Agar, steam until dissolved and then sterilize for twenty minutes at 120° C. A good general agar for Fungi.

4. **Czapek-Dox Agar.**

Sucrose	30 gm.
Sodium nitrate	2 gm.
Potassium phosphate (K_2HPO_4)	1 gm.
Magnesium sulphate	0·5 gm.
Potassium chloride	0·5 gm.
Ferrous sulphate	0·01 gm.
Agar	15 gm.
Water	1,000 c.c.

Sterilize by heating to 100° C. for thirty minutes on three successive days. For cultures of Mucoraceae substitute glucose for sucrose.

5. **Plain Lemco Agar.**

"Lab" Lemco	4 gm.
Peptone	10 gm.
Sodium chloride	5 gm.
Agar	20 gm.
Water	1,000 c.c.

Filter and adjust the reaction to *p*H 7·2. Autoclave for twenty minutes at 120° C. Suitable for growing most Bacteria.

Section 3. Reference Tables

OSMOTIC PRESSURES OF SUCROSE SOLUTIONS IN ATMOSPHERES AT 25° C.
(Ursprung and Blum.) (See also p. 464.)

Molar conc. of solution	0·01	0·05	0·10	0·15	0·20	0·25	0·30
Osmotic pressure	0·25	1·45	2·65	3·92	5·15	6·37	7·73

SORENSEN PHOSPHATE BUFFER MIXTURES

*p*H.	Basic Salt.	Acid Salt.
6·0	1·4 c.c.	8·6 c.c.
6·2	2·0 c.c.	8·0 c.c.
6·4	3·0 c.c.	7·0 c.c.
6·6	4·0 c.c.	6·0 c.c.
6·8	5·0 c.c.	5·0 c.c.
7·0	6·1 c.c.	3·9 c.c.
7·2	7·0 c.c.	3·0 c.c.
7·4	7·8 c.c.	2·2 c.c.
7·6	8·5 c.c.	1·5 c.c.
7·8	9·1 c.c.	0·9 c.c.

Basic salt	M/15 solution of Dibasic sodium phosphate ($Na_2HPO_4\ 2H_2O$) containing 11·876 gm. in 1 litre of solution.
Acid salt	M/15 solution of Monobasic potassium phosphate (KH_2PO_4) containing 9·078 gm. in 1 litre of solution.

Table for the Conversion of Degrees Centigrade into Degrees Fahrenheit and Vice Versa

° C.	° F.	° C.	° F.	° C.	° F.	° C.	° F.	° C.	° F.	° C.	° F.
0	32·0	17	62·6	34	93·2	51	123·8	68	154·4	85	185·0
1	33·8	18	64·4	35	95·0	52	125·6	69	156·2	86	186·8
2	35·6	19	66·2	36	96·8	53	127·4	70	158·0	87	188·6
3	37·4	20	68·0	37	98·6	54	129·2	71	159·8	88	190·4
4	39·2	21	69·8	38	100·4	55	131·0	72	161·6	89	192·2
5	41·0	22	71·6	39	102·2	56	132·8	73	163·4	90	194·0
6	42·8	23	73·4	40	104·0	57	134·6	74	165·2	91	195·8
7	44·6	24	75·2	41	105·8	58	136·4	75	167·0	92	197·6
8	46·4	25	77·0	42	107·6	59	138·2	76	168·8	93	199·4
9	48·2	26	78·8	43	109·4	60	140·0	77	170·6	94	201·2
10	50·0	27	80·6	44	111·2	61	141·8	78	172·4	95	203·0
11	51·8	28	82·4	45	113·0	62	143·6	79	174·2	96	204·8
12	53·6	29	84·2	46	114·8	63	145·4	80	176·0	97	206·6
13	55·4	30	86·0	47	116·6	64	147·2	81	177·8	98	208·4
14	57·2	31	87·8	48	118·4	65	149·0	82	179·6	99	210·2
15	59·0	32	89·6	49	120·2	66	150·8	83	181·4	100	212·0
16	60·8	33	91·4	50	122·0	67	152·6	84	183·2		

Relative Humidity

Conversion of Readings of Wet and Dry Bulb Thermometer

Temp. ° C.	Depression of Wet-Bulb Thermometer in ° C.									
	1·0	2·0	3·0	4·0	5·0	6·0	7·0	8·0	9·0	10·0
+ 0	81	64	46	29	13	...	...	...	...	...
+ 3	84	69	54	40	25	12	...	...	...	...
+ 6	87	73	60	47	35	23	11	...	...	...
+ 9	88	76	65	53	42	32	22	12	...	...
+12	89	78	68	58	48	38	30	21	12	4
+15	90	80	71	62	53	44	36	28	20	13
+18	90	82	73	65	57	49	42	35	27	20
+21	91	83	75	67	60	53	46	39	32	26
+24	92	85	77	70	63	56	49	43	37	31
+27	93	86	79	72	65	59	53	47	41	36
+30	93	86	79	73	67	61	55	50	44	39

OSMOTIC POTENTIALS AT 15° C. IN ATMOSPHERES

	Molarity								
	0·01	0·02	0·05	0·1	0·2	0·3	0·5	0·7	1·0
Sod. chloride .	0·46	0·91	2·24	4·45	8·73	13·0	21.7	29·9	43.1
Pot. chloride .	0·46	0·91	2·18	4·38	8·63	12·4	21·1	29·2	41·4
Pot. nitrate .	0·46	0·90	2·18	4·22	8.04	11·2	18.35	24·6	32·6
Sucrose . .	0·24	0·49	1·40	2·56	4·96	7·49	12·4	17·3	25·0

TABLE OF ATOMIC WEIGHTS OF SOME COMMON ELEMENTS

Element.	Symbol.	Atomic Weight.	Valency.
Aluminium . .	Al	27·0	3
Arsenic . . .	As	74·9	3 and 5
Barium . . .	Ba	137·4	2
Bismuth . . .	Bi	209·0	3 and 5
Boron . . .	B	10·8	3
Bromine . . .	Br	79·9	1
Cadmium . . .	Cd	112·4	2
Calcium . . .	Ca	40·1	2
Carbon . . .	C	12·0	4
Chlorine . . .	Cl	35·5	1
Chromium . .	Cr	52·0	3 and 6
Cobalt . . .	Co	58·9	2 and 3
Copper . . .	Cu	63·6	1 and 2
Fluorine . . .	F	19·0	1
Gold . . .	Au	197·2	3
Hydrogen . . .	H	1·0	1
Iodine . . .	I	126·9	1
Iron	Fe	55·8	2 and 3
Lead . . .	Pb	207·2	2
Lithium . . .	Li	6·9	1
Magnesium . .	Mg	24·3	2
Manganese . .	Mn	54·9	2 and 4
Mercury . . .	Hg	200·6	1 and 2
Nickel . . .	Ni	58·7	2 and 3
Nitrogen . . .	N	14·0	3 and 5
Osmium . . .	Os	190·2	6
Oxygen . . .	O	16·0	2
Phosphorus . .	P	30·2	3 and 5
Platinum . . .	Pt	195·2	2 and 4
Potassium . . .	K	39·1	1
Silicon . . .	Si	28·1	4
Silver . . .	Ag	107·9	1
Sodium . . .	Na	23·0	1
Strontium . . .	Sr	87·6	2
Sulphur . . .	S	32·1	2
Tin	Sn	118·7	2 and 4
Zinc	Zn	65·4	2

Section 4. Addresses of Scientific Suppliers in Great Britain.

A. General Apparatus, Glassware, etc.

A. Gallenkamp & Co., 17-29 Sun Street, Finsbury Square, London, E.C.1.

Baird and Tatlock (London) Ltd., 14-17 St Cross Street, Hatton Gardens, London, E.C.1.

Stanley Belcher and Mason Ltd., Church Street, Birmingham, 3.

Griffin and Tatlock Ltd., Kemble Street, Kingsway, London, W.C.2.

W. and J. George Ltd., 157 Great Charles Street, Birmingham, 3.

Flatters and Garnett Ltd., 309 Oxford Road, Manchester, 13.

B. Chemicals (in addition to the general supplies).

British Drug Houses Ltd., Poole, Dorset.

General Chemical and Pharmaceutical Co. Ltd., Judex Works, Sudbury, Middlesex.

Hopkins and Williams Ltd., 16 St Cross Street, Hatton Gardens, London, E.C.1.

C. Stains and Indicators.

Edward Gurr Ltd., 42 Upper Richmond Road, East Sheen, London, S.W.14.

George T. Gurr, 136 New King's Road, Fulham, London, S.W.6.

D. Microscopes, etc.

Charles Baker, 244 High Holborn, London, W.C.1.

R. and J. Beck Ltd., 69 Mortimer Street, London, W.C.1.

W. R. Prior & Co., 13 North Gate End, Bishops Stortford, Essex.

James Swift & Co. Ltd., 81 Tottenham Court Road, London, W.1.

W. Watson and Sons Ltd., 313 High Holborn, London, W.C.1.

Cook, Troughton and Sims Ltd., Kingsway North, York.

Spencer Lens Co., c/o Hawksley and Sons Ltd., 17 New Cavendish Street, London, W.1.

Bausch and Lomb Optical Co. Ltd., Africa House, Kingsway, London, W.C.2.

E. Botanical Material.

Biological Supply Association, Rhydyfelin, Aberystwyth, Wales.

FOUR-FIGURE LOGARITHMS

	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170						4	9	13	17	21	25	30	34	[illegible]
						0212	0253	0294	0334	0374	4	8	12	16	20	24	28	32	[illegible]
11	0414	0453	0492	0531	0569						4	8	12	15	19	23	27	31	[illegible]
						0607	0645	0682	0719	0755	4	7	11	15	18	22	26	30	[illegible]
12	0792	0828	0864	0899	0934	0969					4	7	11	14	18	21	25	28	32
							1004	1038	1072	1106	3	7	10	14	17	20	24	27	31
13	1139	1173	1206	1239	1271						3	7	10	13	16	20	23	26	30
						1303	1335	1367	1399	1430	3	6	9	13	16	19	22	25	[illegible]
14	1461	1492	1523	1535							3	6	9	12	15	18	21	24	27
					1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27
15	1761	1790	1818	1847	1875	1903					3	6	9	11	14	17	20	23	26
							1931	1959	1987	2014	3	6	8	11	14	17	19	22	25
16	2041	2068	2095	2122	2148						3	5	8	11	13	16	19	21	24
						2175	2201	2227	2253	2279	3	5	8	10	13	16	18	21	23
17	2304	2330	2355	2380	2405	2430					3	5	8	10	13	15	18	20	23
							2455	2480	2504	2529	2	5	7	10	12	15	17	20	22
18	2553	2577	2601	2625	2648						2	5	7	10	12	14	17	19	21
						2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21
19	2788	2810	2833	2856	2878						2	5	7	9	11	14	16	18	20
						2900	2923	2945	2967	2989	2	4	7	9	11	13	15	18	20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11
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37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10
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41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9
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46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	7	8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	5	6	7	8
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52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
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57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
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61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
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67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6
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69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
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73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
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79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
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82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5
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90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
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96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
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ANTILOGARITHMS

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·06	1148	1151	1153	1156	1159	1161	1164	1167	1169	1172	0	1	1	1	1	2	2	2	2
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·82	6607	6622	6637	6653	6668	6683	6699	6714	6730	6745	2	3	5	6	8	9	11	12	14
·83	6761	6776	6792	6808	6823	6839	6855	6871	6887	6902	2	3	5	6	8	9	11	13	14
·84	6918	6934	6950	6966	6982	6998	7015	7031	7047	7063	2	3	5	6	8	10	11	13	15
·85	7079	7096	7112	7129	7145	7161	7178	7194	7211	7228	2	3	5	7	8	10	12	13	15
·86	7244	7261	7278	7295	7311	7328	7345	7362	7379	7396	2	3	5	7	8	10	12	13	15
·87	7413	7430	7447	7464	7482	7499	7516	7534	7551	7568	2	3	5	7	9	10	12	14	16
·88	7586	7603	7621	7638	7656	7674	7691	7709	7727	7745	2	4	5	7	9	11	12	14	16
·89	7762	7780	7798	7816	7834	7852	7870	7889	7907	7925	2	4	5	7	9	11	13	14	16
·90	7943	7962	7980	7998	8017	8035	8054	8072	8091	8110	2	4	6	7	9	11	13	15	17
·91	8128	8147	8166	8185	8204	8222	8241	8260	8279	8299	2	4	6	8	9	11	13	15	17
·92	8318	8337	8356	8375	8395	8414	8433	8453	8472	8492	2	4	6	8	10	12	14	15	17
·93	8511	8531	8551	8570	8590	8610	8630	8650	8670	8690	2	4	6	8	10	12	14	16	18
·94	8710	8730	8750	8770	8790	8810	8831	8851	8872	8892	2	4	6	8	10	12	14	16	18
·95	8913	8933	8954	8974	8995	9016	9036	9057	9078	9099	2	4	6	8	10	12	15	17	19
·96	9120	9141	9162	9183	9204	9226	9247	9268	9290	9311	2	4	6	8	11	13	15	17	19
·97	9333	9354	9376	9397	9419	9441	9462	9484	9506	9528	2	4	7	9	11	13	15	17	20
·98	9550	9572	9594	9616	9638	9661	9683	9705	9727	9750	2	4	7	9	11	13	16	18	20
·99	9772	9795	9817	9840	9863	9886	9908	9931	9954	9977	2	5	7	9	11	14	16	18	20
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9

These Tables are taken from "Tables of Physical and Chemical Constants," by G. W. C. Kaye and T. H. Laby, published by Longmans, Green and Co. Ltd.

INDEX OF PLANT MATERIAL REFERRED TO IN THIS BOOK

Acacia dealbata, 243
— *melanoxylon*, 445
Acer campestre, 223, 232
— *pseudo-platanus*, 189, 207, 208, 210, 221, 232
Aconitum napellus, 224, 263, 273
Aesculus hippocastanum, 196, 197, 214, 431
Aglaozonia, 73
Agropyron repens, 191, 204
Alisma plantago-aquatica, 247
Allium cepa, 32, 34, 214, 260, 262
— — var. *bulbiferum*, 410
Aloe sp., 212
Althaea rosea, 265
Amanita phalloides, 102
Ammophila arenaria, 47, 191, 384
Ampelopsis veitchii, 219, 394
Anabaena azollae, 147
— *cycadacearum*, 181
Anagallis arvensis, 34, 264
Ananas sativa, 266
Anthoceros laevis, 115
Anthriscus sylvestris, 248, 280
Apium graveolens, 52
Aquilegia sp., 263
Arabis, 233
Arctium minus, 268
Arcyria denudata, 83
Aristolochia sipho, 200, 201
Arrhenatherum elatius, 243, 252, 290
Arum maculatum, 250, 287
Ascophyllum nodosum, 399
Asparagus plumosus, 194
Aspergillus, 92
Asperula odorata, 223
Asplenium bulbiferum, 411
Aster sp., 194
— *novae-angliae*, 409
Aubrieta, 233
Aucuba japonica, 35, 223
Avena sativa, 224, 229
Azolla filiculoides, 145

Bacillus subtilis, 106
Batrachospermum, 65
Begonia sp., 43
— *rex*, 51
Bellis sp., 248
Berberis darwini, 250
— *stenophylla*, 250
— *vulgaris*, 222
Bertholletia excelsa, 44
Beta maritima, 390
— *vulgaris*, 35, 51, 390
Betula pendula, 268
Bidens cernua, 268
Brassica napus, 195
— *oleracea* var. *botrytis*, 195
— — — *capitata*, 195
Brassica oleracia var. *gemmifera*, 197
Botrychium lunaria, 144
Bryophyllum calycinum, 412

Calluna vulgaris, 402
Caltha palustris, 238, 244, 269, 273
Calystegia sepium, 392
Camellia japonica, 51
Capsella, 233
— *bursa-pastoris*, 224, 254, 450
— — var. *integrifolia*, 450
— — — *taraxacifolia*, 450
Cardamine bulbifera, 411
Carpinus betulus, 268
Catharinea undulata, 39
Cerastium sp., 248
Chara, 108
Cheiranthus, 233
— *cheiri*, 274
Chelidonium majus, 443
— — var. *laciniatum*, 443
Chelone barbata, 267
Chlamydomonas, 54
Chondrus crispus, 66
Choreocolax polysiphoniae, 400
Chrysanthemum leucanthemum, 240, 249
— *maximum*, 249
Circaea lutetiana, 43
Citrus limonum, 236
Claviceps purpurea, 97
Clematis vitalba, 267, 392, 394
Cobaea scandens, 393, 394
Cocos nucifera, 269
Collomia cocinea, 47
Convallaria majalis, 192, 203, 225, 248
Corallina officinalis, 69
Cordyline australis, 212
Corylus avellana, 286
Cotoneaster horizontalis, 431
Crambe maritima, 413
Crataego-mespilus, 453
Crataegus monogyna, 453
— *oxyacanthoides*, 195
Crocus sp., 193
— *aureus*, 391
Cucurbita sp., 47
— *pepo*, 199, 217, 218, 393
Cuscuta epithymum, 396
Cutleria multifida, 72
Cyathus striatus, 104
Cycas circinalis, 182
— *revoluta*, 177
Cystococcus humicola, 402
Cystopus candidus, 90
Cytisus purpureus, 451

Dahlia variabilis, 42, 49, 214
Daucus carota, 40, 195
Delphinium sp., 263

Dentaria bulbifera, 411
Dictyota, 74
Digitalis purpurea, 239, 284
Dionaea muscipula, 408
Doronicum plantagineum, 280
Draba verna, 448
Dracaena sp., 212
Drosera rotundifolia, 234, 406
Dryopteris filix-mas, 131

Ectocarpus confervoides, 71
Elodea canadensis, 33, 39, 387, 411
Ephedra, 187
Epidendrum sp., 220
Epilobium montanum, 267
Equisetum arvense, 149, 439
Eranthis hiemalis, 438
Erica cinerea, 249, 282, 382
— *tetralix*, 249
Eriophila verna, 448
Erysiphe, 93
Eschscholtzia californica, 253
Eucalyptus globulus, 236
Eudorina, 56
Euonymus sp., 248
Euphorbia splendens, 237

Fagus sylvatica, 48, 221, 223, 386
— — var. *cuprea*, 443
— — — *heterophylla*, 443
Fegatella conica, 39
Festuca ovina var. *vivipara*, 409
Ficus carica, 266
— *elastica*, 44
Forsythia suspensa, 197
Fragaria vesca, 194, 266, 278
Fraxinus excelsior, 224, 263, 268
Fuchsia macrostemma, 227
— *riccartoni*, 227, 244
Fucus serratus, 78
— *spiralis*, 78
— *vesiculosus*, 78
Fuligo septica, 82, 84
Funaria hygrometrica, 39, 122

Galium aparine, 268, 393
Geranium robertianum, 267, 276
Geum intermedium, 448
— *rivale*, 448
— *urbanum*, 268, 448
Ginkgo biloba, 184
Gladiolus sp., 193
— *primulinus*, 391
Grammatophyllum, 220

Haematococcus, 55
Hakea pectinata, 52, 383
Harpalium, 409
Harveyella mirabilis, 400
Hedera helix, 214, 224, 236
Helianthus annuus, 226
— *rigidus*, 194, 200, 408
— *tuberosus*, 192
Helleborus niger, 238
Heracleum sphondylium, 248, 264
Hippuris vulgaris, 205, 388
Humulus lupulus, 234, 268, 391
Hyacinthus orientalis, 43
Hydrangea vulgaris, 202
Hydrocharis morsus-ranae, 412
Hymenophyllum tunbridgense, 142
Hypoxylon coccineum, 98

Iberis, 248
Ilex aquifolium, 47, 228
Iris foetidissima, 34
— *germanica*, 192, 230
— *pseudacorus*, 34
Isoetes lacustris, 160

Juncus effusus, 389

Laburnum adami, 451
— *anagyroides*, 53, 211, 248, 451
Laminaria cloustoni, 76
— *digitata*, 76
— *saccharina*, 75
Lamium album, 34, 41, 202, 240, 285
Lathraea squamaria, 41, 42, 398
Lathyrus aphaca, 394
Lavandula vera, 382
Lemna minor, 43, 214
Lepidium sativum, 217
Ligustrum vulgare, 39, 226
Lilium sp., 241, 243, 246
— *candidum*, 228
Linaria repens, 409
Lonicera periclymenum, 392
Lotus corniculatus, 374
Lupinus polyphyllus, 224
Lycogala epidendrum, 84
Lycopersicum esculentum, 40, 265
Lycopodium clavatum, 154

Malva sp., 242
Marchantia polymorpha, 112
Matthiola incana, 233, 264
Melandrium dioicum, 264
Mentha sp., 194
— *spicata*, 193
— *viridis*, 409
Mespilus germanica, 453
Mimosa pudica, 378
Mnium hornum, 39, 117
Monoblepharis sphaerica, 87
Monotropa hypopitys, 402
Mucor, 84
— *mucedo*, 438
Mutisia ilicifolia, 394
Myosotis sp., 248, 268
Myriophyllum spicatum, 44
— *verticillatum*, 387
Myrtus communis, 235

Narcissus sp., 193, 254
— *majalis*, 229
— *pseudo-narcissus*, 289
Neottia nidus-avis, 402
Nepenthes destillatoria, 404

Nepenthes villosa, 404
Nicotiana tabacum, 229
Nostoc, 65, 401
Nymphaea alba, 385

Oedogonium, 63
Oenanthe crocata, 269
Oenothera sp., 242
— *biennis*, 263
Opuntia sp., 196
Orchis sp., 261
— *mascula*, 287
Orobanche apiculata, 398
— *minor*, 398
Osmunda regalis, 143
Oxalis acetosella, 374

Pandorina, 56
Papaver dubium, 264
— *rhoeas*, 273
Parietaria diffusa, 41, 44
Passiflora coerulea, 395
Pedicularis sylvatica, 399
Pelargonium zonale, 39, 40, 230
Pellia epiphylla, 109
Pellionia sp., 41
Peltigera canina, 400
Peronospora, 91
Peziza, 94
Phajus sp., 41
Phallus impudicus, 105
Phaseolus multiflorus, 50, 201, 231, 233, 391
— *vulgaris*, 258, 260
Phormium tenax, 384
Pinguicula vulgaris, 235, 406
Pinus sylvestris, 164
Pisum sativum, 263, 277, 394, 433, 447
Plantago coronopus, 450
— *lanceolata*, 252
— *media*, 215, 248, 252
Plasmodiophora brassicae, 81
Platanus orientalis, 233
Pleurococcus, 59
Poa alpina, 410
Polygonatum multiflorum, 191
Polygonum viviparum, 410
Polyporus betulinus, 102
Polysiphonia, 67
— *fastigiata*, 399
Polytrichum commune, 124
— *juniperinum*, 124
— *piliferum*, 124
Populus alba, 409
— *nigra*, 228
— *serotina*, 228
Potamogeton natans, 388
Potentilla anserina, 194
— *reptans*, 194, 224
Primula sp., 34
— *vulgaris*, 233, 239, 251, 283
Prunus cerasus, 190, 239, 248, 265
— *domestica*, 190
— *lauro-cerasus*, 223, 232
Psalliota campestris, 102
Pteridium aquilinum, 139
Pteris serrulata, 142
Puccinia graminis, 99
Pyrus communis, 51, 221, 239, 248
— *domestica*, 190
— *malus*, 190, 239, 266, 279
Pythium debaryanum, 89

Quercus robur, 223, 252

Ranunculus acris, 238, 262, 272
— *bulbosus*, 213
— *ficaria*, 41
— *repens*, 213, 215, 216, 224
Reticularia lycoperdon, 83, 84
Rhododendron sp., 282
— *ponticum*, 227
Rhytisma acerinum, 95
Ribes rubrum, 221
— *sanguineum*, 225
— *uva-crispa*, 225, 265
Ricinus communis, 45, 258, 261
Rosa sp., 40, 52, 224, 225, 239
Rubus fruticosus, 393, 411
— *idaeus*, 409
Ruscus aculeatus, 194, 203

Saccharomyces cerevisiae, 96
Salicornia stricta, 389
Salix sp., 248, 413
— *atrocinerea*, 286
— *caprea*, 286
Salvia dichroa, 251
Sambucus nigra, 31, 50, 222, 224
Saprolegnia, 86
Sarothamnus scoparius, 196, 250, 263, 386
Saxifraga umbrosa, 279
Scilla hispanica, 249
— *non-scripta*, 43, 243, 289
Scrophularia nodosa, 263
Sedum, 248
Selaginella kraussiana, 156
Sempervivum tectorum, 383, 409
Senecio vulgaris, 268
Sinapis alba, 217
Solanum tuberosum, 41, 49, 192, 283
Spartium junceum, 387
Sphagnum, 129
Spirogyra, 60
Staphylococcus albus, 107
— *aureus*, 107
Stellaria holostea, 248, 275
Stemonitis splendens, 84
Symphoricarpus racemosus, 32
Syringa vulgaris, 196, 223, 225, 232

Tamus communis, 40
Taraxacum officinale, 42, 49, 50, 213, 224, 236, 248, 262, 281, 414
Taxus baccata, 173, 437, 441
— — var. *fastigiata*, 443
Tilia sp., 47
— *vulgaris*, 197, 208, 209
Tradescantia virginica, 33
— *zebrina*, 230
Tragopogon porrifolius, 268
— *pratensis*, 267
Trichia varia, 84

Trifolium sp., 402
— *pratense*, 224, 374
Triticum vulgare, 42, 44, 49, 261, 262
Tritonia crocosmiaeflora, 43, 56
Tropaeolum majus, 198, 204, 224
Tulipa sp., 193, 288
Tussilago farfara, 51, 191

Ulex europaeus, 195, 278, 387
Ulmus hollandica, 222
— *procera*, 197, 218, 263, 268, 409
Ulothrix, 59
Urtica dioica, 235
Utricularia vulgaris, 403

Vaccinium macrocarpum, 265
Vanda sp., 220
— *teres*, 395
Vaucheria, 61
Verbascum thapsus, 233
Veronica sp., 223
Vicia faba, 35, 36, 189, 202, 213, 216, 257, 258, 260, 390, 433
— *sativa*, 445
Viola canina, 239, 275
— *lactea*, 449
— *odorata*, 239, 266
— *riviniana*, 449
Viscum album, 201, 397
Vitis sp., 47
— *inconstans*, 394
Volvox, 58

Xanthoria parietina, 401

Yucca sp., 212
— *gloriosa*, 47

Zea mais, 204, 214, 231, 253, 259, 262, 446
Zebrina pendula, 230

INDEX OF SELECTED SUBJECTS

(*To be used in conjunction with Table of Contents, pp. vii-xviii*)

Aggregate species, 448
Algae, herbarium specimens, 436
Amoeba, artificial, 300
Antagonism, 310

Bacteria, cultivation of, 106
Barcroft (Thoday) method (respiration), 352
Barger's method (osmotic potential), 305
Bedell's auxanometer, 363
Biuret test, 48
Boysen Jensen method (respiration), 347

Callose, 47
Catalase, 346
Chromatogram, 299, 339
Chromatography, 298, 339
Clearing, 16
Coagulability, 296
Coal gas, passage through wood, 325
Colour filters, 328, 373
Contractile root, 391
Corpus, 205
Correlation coefficient, 440
Culture media, 81, 461

Darwin potometer, 313
Dehydrogenase, 347
Differential thermometer, 319
Double staining, 16
Drying paper, herbarium, 434

Elementary species, 448
Erepsin, 345
Evaporimeter, 429

Farmer's auxanometer, 361
— potometer, 316
Fungi, cultivation of, 80
—, herbarium specimens, 436

Ganong disc method (photosynthesis), 337
— markers, 360, 361
— photosynthometer, 335
— respirometer, 349
Genetical experiments, 447
Gram stain, 106
Growth-promoting substances, 369

Hemicellulose, 46
Herbarium paper, 434
Heterauxin, 369

Indicators, 291, 460
Indigo-carmine method, 333
Infiltration, 28
Intra-vitam staining, 25
Inulin, 42, 49
Isotonic concentration, 306

Jordanon, 448

Kolkwitz' method (photosynthesis), 333

Latex, 50, 236
Leslie's differential thermometer, 319
Lignin, 46
Linneon, 448

McDougall's respiroscope, 348
Micro-species, 448
Millon's reagent, 48
Moll's experiment (absorption of carbon dioxide), 330
Molz' method (suction force), 308
Mucilage, 47
Multiple cambium, 390

Neilson Jones' method (respiration), 347
— — root auxanometer, 363

Papain, 344
Pectic substances, 47
Pepsin, 344
Peroxidase, 346
Plants, drying of, 434
Polarity, 369, 413
Priestley's experiment (photosynthesis), 335
Prime's method (photosynthesis), 332
Protonema, 122

Rennet, 299
Resin, 48
Respiroscope, 348

Sach's half-leaf method, 337
Saponin, 300

Seeds, swelling of, 297
Silica, 47
Smear preparations, 22
Soil texture, 421
Spodogram, 48
Standard deviation, 441
Stem succulents, 196
Stinging hair, 235
Suberin, 46
Sugars, 49
Surface film, 300
Switch plant, 196

Tannin, 48
Temperature coefficient, 364
Thoday's potometer, 315
Traube's cell, 304
Tswett's chromatogram, 339
Tunica, 205
Turion, 412
Tyndall effect, 294

Velamen, 395

Xanthoproteic test, 48

Yeast, 357

Zymin, 358

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